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Systematics of the Lizard Family Pygopodidae with Implications for the Diversification of Australian Temperate Biotas

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Abstract.— We conducted a phylogenetic study of pygopodid lizards, a group of 38 species endemic to Australia and New Guinea, with two major goals: to reconstruct a taxonomically complete and robustly supported phylogeny for the group and to use this information to gain insights into the tempo, mode, and timing of the pygopodid radiation. Phylogenetic analyses of mitochondrial DNA (mtDNA), nuclear DNA (nDNA), and previously published morphological data using parsimony, maximum likelihood, and Bayesian methods on the independent and combined three data sets yielded trees with similar and largely stable ingroup topologies. However, relationships among the six most inclusive and unambiguously supported clades (*Aprasia*, *Delma*, *Lialis*, *Ophidiocephalus*, *Pletholax*, and *Pygopus*) varied depending on data set analyzed. We used parametric bootstrapping to help us understand which of the three-branch schemes linking these six taxa was most plausible given our data. We conclude based on our results that the arrangement ((((*Delma*, *Lialis*)*Pygopus*)*Pletholax*)(*Aprasia*, *Ophidiocephalus*)) represents the best hypothesis of intergeneric relationships. A second major problem to arise in our study concerned the inability of our two outgroup taxa (*Diplodactylus*) to root trees properly; three different rooting locations were suggested depending upon analysis. This long-branch attraction problem was so severe that the outgroup branch also interfered with estimation of ingroup relationships. We therefore used the molecular clock method to root the pygopodid tree. Results of two independent molecular clock analyses (mtDNA and nDNA) converged upon the same root location (branch leading to *Delma*). We are confident that we have found the correct root because the possibility of our clock estimates agreeing by chance alone is remote given that there are 65 possible root locations (branches) on the pygopodid tree (~1 in 65 odds). Our analysis also indicated that *Delma fraseri* is not monophyletic, a result supported by a parametric bootstrapping test. We elevated the Western Australian race, *Delma f. petersoni*, to species status (i.e., *Delma petersoni*) because hybridization and incomplete lineage sorting could be ruled out as potential causes of this paraphyletic gene tree and because *D. grayii* is broadly sympatric with its sister species *D. fraseri*. Climate changes over the past 23 million years, which transformed Australia from a wet, green continent to one that is largely dry and brown, have been suspected as playing a major role in the diversification of Australia's temperate biotas. Our phylogenetic analyses of pygopodid speciation and biogeography revealed four important findings consistent with this climate change diversification model: (1) our fossil-calibrated phylogeny shows that although some extant pygopodid lineages predate the onset of aridification, 28 of 33 pygopodid species included in our study seem to have originated in the last 23 million years; (2) relative cladogenesis tests suggest that several major clades underwent higher than expected rates of speciation; (3) our findings support earlier studies showing that speciation of mesic-adapted biotas in the southeastern and southwestern corners of Australia largely occurred within each of these regions between 12 and 23 million years ago as opposed to repeated dispersal between these regions; and (4) we have identified for the first time the existence of several pairs of sympatric sister species of lizards living in arid and semiarid ecosystems. These sympatric sister species seem to be younger than allopatric or parapatric sister-species pairs, which is not consistent with previous beliefs. [Australia; biogeography; lizard; molecular clock; parametric bootstrapping; phylogeny; pygopodidae; tree rooting.]

Australia experienced an explosion in lizard diversity as all families of lizards found on this continent, including Scincidae, Varanidae, Agamidae, Gekkonidae, and Pygopodidae, underwent radiations (Cogger, 1992; Ehmann, 1992). Lizard diversity is particularly astonishing in the arid and semiarid regions of Australia, where 40–50 species can be found in local sympatry (Pianka, 1986; Morton and James, 1988), a phenomenon that has attracted considerable attention by community ecologists interested in understanding how these diverse assemblages are maintained (Pianka, 1969, 1986, 1989; Morton and James, 1988). Unfortunately, we still know little about why or when these radiations occurred, a problem that stems from a lack of well-corroborated phylogenies for these groups.

A distinctive element of Australia's lizard fauna is the family Pygopodidae, a group of limb-reduced, elongate lizards endemic to Australia and New Guinea (see Photo 1; Kluge, 1974, 1976; Cogger, 1992; Ehmann, 1992; Shea, 1993; Smith and Henry, 1999). At present, 38 species of pygopodids are recognized within six to eight genera (Kluge, 1974, 1976; Cogger, 1992; Ehmann, 1992; Shea, 1993; Smith and Henry, 1999; James et al., 2000). Pygopodid phylogeny was first investigated by Kluge (1976), who inferred relationships among 21 extant species using 86 morphological characters (Fig. 1). Kluge's analysis garnered strong support for only four clades (*Aprasia*, *Delma*, *Lialis*, and *Pygopus*) and two single-species lineages (*Ophidiocephalus taeniatus* and *Pletholax gracilis*), but placement of the root in Kluge's tree actually renders *Pygopus* paraphyletic (Fig. 1). Despite this finding, Kluge still considered *Pygopus* as a monophyletic group, implying that placement of the root in his tree is questionable.

As to the affinities of pygopodids to other squamates, this group is clearly derived from a stock of gekkonid lizards (Underwood, 1957; Kluge, 1974, 1976, 1987; Estes et al., 1988; Shea, 1993; Donnellan et al., 1999). However, exactly which extant group of gekkonids is most closely related to pygopodids has been controversial. Kluge (1987), based on his morphological phylogenetic study of

PHOTO 1. The Javelin Lizard, *Delma concinna*, of Western Australia is one of about 38 extant species of pygopodids. This species was originally given the generic name of "Aclys," meaning "short javelin" in Latin (Kluge, 1974), in recognition of its spear-like morphology. Photo by W. Bryan Jennings.

the Gekkonoidea, proposed that Australian diplodactyline geckos are the sister group to pygopodids. However, Estes et al. (1988) were skeptical of Kluge's hypothesis and proposed an alternative hypothesis, one that places the pygopodids as sister to all other geckos. The idea that pygopodids and diplodactyline geckos are sister groups is now also supported by a molecular phylogenetic study (Donnellan et al., 1999).

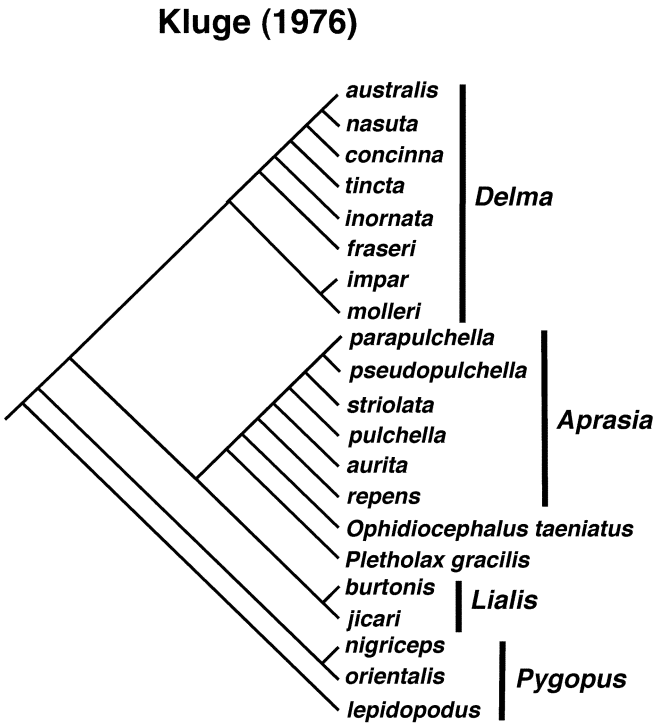

FIGURE 1. Hypothesized relationships among 21 species of pygopodid lizards inferred from morphological data (after Kluge, 1976).

The origins of Australia's fauna and flora can be attributed to Gondwanan vicariance or dispersal from Asia (Storr, 1964). Although some squamate groups presently found in Australia such as colubrid snakes and agamid lizards are descendents of the Asiatic fauna (Shine, 1991), pygopodids clearly have a Gondwanan history owing to their endemicity (Kluge, 1974; Wilson and Knowles, 1988; Greer, 1989; Cogger, 1992; Ehmann, 1992; Shea, 1993), probable derivation from a Gondwanan gecko lineage (i.e., ancestors of diplodactyline geckos; Kluge, 1987; Greer, 1989; Donnellan et al., 1999), and fossil evidence that establishes their presence in Australia well before the time when Asian faunal and floristic elements invaded Australia (Hutchinson, 1997).

Here, we present for the first time a molecular perspective of pygopodid phylogeny using both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) sequences. We also reanalyzed Kluge's morphological data in conjunction with our molecular data. Such an analysis may not only reveal patterns that reflect how pygopodids diversified but, of broader significance, may also provide insights that may help explain other radiations in Australia.

MATERIALS AND METHODS

Taxon Sampling

We obtained tissue samples of 32 of the 38 known pygopodid species and two subspecific taxa (Table 1). This sample includes representatives of all putative genera (i.e., *Aclys*, *Aprasia*, *Delma*, *Lialis*, *Ophidiocephalus*, *Paradelma*, *Pletholax*, and *Pygopus*). Missing from this study are *Aprasia haroldi*, *A. rostrata*, *Delma elegans*, *D. plebeia*, *Pygopus schraderi*, and *P. steelescotti*. Throughout this paper, *Aclys concinna* (Kluge, 1974) is included as a species of *Delma* (Kluge, 1976), and similarly *Paradelma orientalis* (Kluge, 1974) is treated as a species of *Pygopus* (Kluge, 1976). Two species of diplodactyline geckos, *Diplodactylus damaeus* and *D. tessellatus*, members of the sister group, were included as outgroup lineages. Tissue samples of muscle, liver, or blood were preserved in 95% ethanol, a 1:1 solution of 7% saline and 70% ethanol, or a tissue storage buffer consisting of 250 mM EDTA (pH 7.5), 20% DMSO, and saturated NaCl.

Molecular Data

Genomic DNA was extracted following standard phenol/chloroform extraction methods (Maniatis et al., 1982) or by using QIAQuick DNA extraction kits (Qiagen). We followed general polymerase chain reaction (PCR) procedures for obtaining DNA sequence data as outlined by Palumbi (1996) and Hillis et al. (1996a). All PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) or gel purified

TABLE 1. Taxa, locality data, and sources of genetic material included in this study.

Taxon[a]	Locality[b]	Collection no.[c]
Aprasia aurita	Wathe Fauna Reserve, VIC	SAM-R43054
Aprasia fusca	1 km NW Bullara Homestead, WA	SAM-AR52288
Aprasia inaurita	St. Peter Island, SA	SAM-R47087
Aprasia parapulchella	Bendigo Whipstick, VIC	MV-D66569
Aprasia picturata	35 km E Leonora, WA	WAM-R131647
Aprasia pseudopulchella	2 km E Burra, SA	SAM-R40729
Aprasia pulchella	Jarrahdale, WA	WAM-R80000
Aprasia repens	Booragoon, WA	WAM-R106018
Aprasia smithi	WA (no other data)	SAM-R106018
Aprasia striolata	Flinders Island 10 km NW Port Lincoln, SA	ABTC-6575
Delma australis	Mt. Remarkable, SA	SAM-R22784
Delma borea	Leopold Downs, Halls Creek, WA	ERP-W31365
Delma b. butleri	Coonbah, NSW	SAM-R36144
Delma b. haroldi	Wave Hill Station, NT	NTMR-16484
Delma (*Aclys*) *concinna*	Lesueur National Park, WA	WBJ-2477
Delma f. fraseri	Lesueur National Park, WA	WBJ-1999
Delma f. fraseri	Lesueur National Park, WA	WBJ-2329
Delma f. petersoni	30 km W Buckleboo, SA	SAM-R20804
Delma f. petersoni	Secret Rocks, SA	SAM-R40756
Delma f. petersoni	Yumburra Conservation Park, SA	SAM-R68364
Delma grayii	WA (no other data)	ABTC-6577
Delma grayii	Lesueur National Park, WA	WBJ-2659
Delma impar	Gungahlin Town Center, ACT	SAM-R43328
Delma inornata	Lake Alexandria, SA	SAM-R23530
Delma labialis	Singapore Bay, Keswick Island, QLD	QM-J62835
Delma mitella	7.8 km W Paluma, QLD	ABTC-58998
Delma molleri	Mt. Remarkable, SA	SAM-R23137
Delma nasuta	71 km W Windorah, QLD	SAM-R42914
Delma pax	South Hedland, WA	WAM-R106278
Delma tincta	55 km SE Winton, QLD	SAM-R90213
Delma torquata	Grongah State Forest, QLD	QM-J63361
Lialis burtonis	Laverton, WA	ERP-30061
Lialis jicari	Irian Jaya	JAM
Ophidiocephalus taeniatus	Todmorden Station, SA	SAM-R44653
Pletholax gracilis	Lesueur National Park, WA	WBJ-2483
Pygopus lepidopodus	Lesueur National Park, WA	WBJ-1206
Pygopus nigriceps	Laverton, WA	ERP-R29509
Pygopus (*Paradelma*) *orientalis*	20 km N Capella, QLD	QM-J56089
Diplodactylus damaeus	Laverton, WA	ERP-30330
Diplodactylus tessellatus	Tracking Station beside Stuart Hwy, SA	SAM-R41130

[a]Names in parentheses are alternative generic names recognized by some authorities (e.g., Cogger, 1992; Ehmann, 1992).

[b]ACT = Australian Capital Territory; NSW = New South Wales; NT = Northern Territory; SA = South Australia; WA = Western Australia; QLD = Queensland; VIC = Victoria.

[c]MV = Museum of Victoria; ABTC = Australian Biological Tissue Collection, South Australian Museum; SAM = South Australian Museum; WAM = Western Australian Museum; QM = Queensland Museum; NTMR = Northern Territory Museum; WBJ = W. B. Jennings field number; ERP = E. R. Pianka field number; JAM = J. A. McGuire.

using a QIAquick gel purification kit (Qiagen). Extension products were visualized on a Perkin-Elmer ABI 377 automated sequencer, and all PCR products were sequenced in both directions to safeguard against errors. PCR primers and sequencing primers used in this study are listed in Table 2. Initially, we sequenced two regions of the mitochondrial genome, a 772-base pair (bp) fragment of the 16S ribosomal RNA subunit (16S gene) and a 1,311-bp sequence that included the entire NADH dehydrogenase subunit 2 gene (*ND2*) and the flanking transfer RNA (tRNA) genes methionine (partial), tryptophan (entire), alanine (entire), and asparagine (partial). Preliminary phylogenetic analyses suggested that these markers were only capable of reconstructing relatively shallow relationships and not the deeper branches such as those linking generic lineages. This finding prompted us to incorporate a more conserved marker that might better resolve deeper divergences (e.g., Hillis, 1987; Flook et al., 1999). Recent studies have included the nuclear oncogene *c-mos* to resolve higher level relationships among a wide range of vertebrate groups (Cooper and Penny, 1997; Saint et al., 1998; Donnellan et al., 1999). We therefore included a 417-bp sequence using primers developed by Saint et al. (1998). The same individuals were sequenced for each gene, and all DNA sequences are deposited in GenBank (accession nos. AY134500–AY134607).

Morphological Data

In addition to the molecular data gathered for this study, we also reanalyzed Kluge's (1976) morphological data set containing 86 osteological characters for 21 pygopodid species. Full descriptions of these

TABLE 2. Amplification and sequencing primers (5′ to 3′) used in this study.

Name	Sequence[a]	Position[b]	Source[c]
L4437	AAGCTTTCGGGCCCATA	L4437	1
ND2b	GCCCATACCCCAAAAATGTYG	L4449	2
ND2c	AACCAAACCCAAACACGAAARATYAT	L5005	2
ND2d	AAACCAAGAGCCTTCAAAG	L5549	2
ND2f	TGTRGTTATRTGDGATATYCG	H5352	2
ND2e	GCGCGCTGGTTTGGGTDWTTAGYTGTTAA	H5692	2
16Sc	GTMGGCCTAAAAGCAGCCAC	L2189	3
16Scm	GCGGTATCCTAACCGTGCAAAGG	L2593	2
16Sbm	CCTTTGCACGGTTAGGATACCGC	L2571	2
16Sb	GCGCTGTTATCCCTAGGGTAACTTG	H2920	3
G73	GCGGTAAAGCAGGTGAAGAAA	—	4
G74	TGAGCATCCAAAGTCTCCAATC	—	4

[a]Redundancy codes: Y = C or T; R = A or G; M = A or C; W = A or T; D = A, T, or G.
[b]Positions of the 3′ nucleotides of all primers are given in reference to the complete human mitochondrial genome sequence (Anderson et al., 1981).
[c]Sources of primers: 1 = Macey et al. (1997); 2 = this study; 3 = Reeder (1995); 4 = Saint et al. (1998).

characters, character states, and complete data matrix were provided by Kluge (1976).

Phylogenetic Analyses

Nucleotide sequence alignments.—Protein coding sequences were unambiguously aligned by eye using Se-Al (Rambaut, 1995). However, the positional homology of several insertion/deletion regions located in the noncoding sequences could not be determined; therefore, these hypervariable regions were deleted from the data set. Details on which sites were excluded from analyses were provided by Jennings (2002) and are in Appendix A on the *Systematic Biology* Web site. Mitochondrial DNA and nDNA sequences were separately tested for homogeneity of nucleotide composition among taxa using the χ^2 homogeneity test in PAUP* 4.0.0d64 (Swofford, 2000). Only informative sites were tested because these sites are most influential in tree construction and inclusion of constant sites may obscure significant heterogeneity when it exists. Significant nucleotide bias was observed in the mtDNA sequences ($P = 0.023$) and seemed to be due to the high thymine content in *Pygopus nigriceps*. The nuclear gene did not show such a bias ($P = 0.99$).

Parsimony analyses.—We conducted phylogenetic analyses using the parsimony criterion implemented in PAUP* 4.0.0d64 (Swofford, 2000) on mtDNA, nDNA, mtDNA + nDNA, and mtDNA + nDNA + morphology data sets. All characters were weighted equally. Molecular data were treated as unordered, and morphological characters were ordered. Tree searches were conducted heuristically with tree bisection–reconnection (TBR) branch swapping in a random stepwise addition of taxa repeated 100 times. Clade support was assessed using the nonparametric bootstrap technique (Felsenstein, 1985; Hillis and Bull, 1993) as implemented in PAUP* 4.0.0d64.

Maximum likelihood analyses.—Phylogenetic trees were also estimated under the maximum likelihood (ML) criterion using PAUP* 4.0.0d64. Only the molecular data sets were analyzed. Substitution models for each molecular data set were selected using Modeltest 3.06 (Posada and Crandall, 1998). Once a model was selected for a given molecular data set, we then used this model and its parameter estimates to search for a new optimal tree via heuristic searches of tree space using TBR branch swapping in a random stepwise addition of taxa repeated 10 times. Model parameters were then reoptimized with the newly obtained tree. Tree searches were conducted heuristically with TBR branch swapping in a random stepwise addition of taxa repeated 100 times. Clade support was assessed using the nonparametric bootstrap.

Bayesian analyses.—New Bayesian Markov chain Monte Carlo methods provide a third means by which phylogenetic trees may be estimated (Li, 1996; Mau, 1996; Rannala and Yang, 1996; Mau and Newton, 1997; Yang and Rannala, 1997; Larget and Simon, 1999; Mau et al., 1999; Newton et al., 1999; Huelsenbeck and Ronquist, 2001). These new phylogenetic tools enjoy computational advantages over ML for large data sets (Huelsenbeck et al., 2001) plus have the additional benefit, especially relevant for the present study, of allowing for combined analysis of DNA and morphological characters using mixed models. We analyzed our data sets using MrBayes 3.0B4 (Huelsenbeck and Ronquist, 2001). Nucleotide substitution models used in Bayesian analyses were the same as those applied in likelihood analyses. Priors for parameterization were set as follows: rate matrix = flat Dirichlet (1, 1, 1, 1, 1, 1), state frequencies = Dirichlet, shape parameter = uniform (0.05, 50), Pinvar = uniform (0, 1), transition/transversion rate ratio = flat Beta (1, 1), topology = all topologies equally probable a priori, and branch lengths = unconstrained exponential in nonclock analyses and constrained uniform for clock analyses. In each run, the default setting of four Markov chains was chosen. Beginning from two random starting trees, we ran 1 million generations sampling the Markov chains every 100 generations to obtain 10,000 samples. Graphical analysis of log likelihoods versus generation time suggested that log likelihoods converged to stable values between 9,000 and 21,000 generations into each run, depending upon data set. We therefore discarded the first 1,000 trees in each run as burn-in samples and used the remaining 9,000 trees to construct majority-rule consensus

trees. Bayesian posterior probabilities for each clade were derived from these 9,000 trees. For ease of visual comparison to bootstrap values, we present these probabilities as numbers ranging from 0 to 100 rather than 0 to 1.0. Following other workers (e.g., Leaché and Reeder, 2002; Wilcox et al., 2002), we consider posterior probabilities of $\geq$95 as strong support for a clade's existence.

Inferring the Root of the Pygopodid Tree Using the Molecular Clock Method

Proper rooting of a phylogenetic tree using the outgroup method can be problematic if the outgroup and ingroup are too divergent from each other (Swofford et al., 1996; Huelsenbeck et al., 2002). Although there is strong evidence to support the hypothesis that diplodactyline geckos and pygopodids are each other's closest living relatives (Kluge, 1987; Donnellan et al., 1999), the two groups have undergone extreme levels of morphological and possibly sequence divergence. This raises the possibility that use of either group to root its sister lineage in a phylogenetic analysis may be unproductive. Huelsenbeck et al. (2002) suggested that the molecular clock can be used to root a phylogenetic tree. Following Huelsenbeck et al. (2002), we used MrBayes (Huelsenbeck and Ronquist, 2001) to analyze the mtDNA, nDNA, and combined mtDNA + nDNA data sets in a Bayesian framework with the molecular clock enforced and with outgroups excluded. We tested the molecular clock assumption by comparing clock and nonclock log-likelihood values obtained from PAUP*-scored trees using the likelihood-ratio test (Goldman, 1993). We determined the statistical significance of the test statistic, δ (equal to the difference in log-likelihood scores multiplied by 2), by referring to the χ^2 table of Rohlf and Sokal (1981) and using $N - 2$ ($N =$ number of species) degrees of freedom (Huelsenbeck and Rannala, 1997). Because the molecular clock and outgroup root estimates are independent of each other, conducting both analyses on the same data sets provides an opportunity to find agreement in rooting hypotheses.

Hypothesis Testing

We used parametric bootstrapping to test several phylogenetic hypotheses suggested by the results of this study (Hillis et al., 1996b; Huelsenbeck et al., 1996; also see Wilcox et al., 2002, for similar application of this method). Using PAUP* and the mtDNA + nDNA data set, we conducted a tree search under the parsimony criterion using the model tree that had a constraint consistent with the null hypothesis (i.e., the tree obtained from analysis of the morphology data only). A parsimony analysis was also conducted using the same data but without any constraints. The test statistic is the difference in parsimony tree scores between the best trees found under constrained and those found under unconstrained tree searches. A null distribution in parsimony score differences was generated in the following manner. The best tree consistent with the null hypothesis was scored under an ML criterion using the same model as before (i.e., GTR + Γ + I) so that branch lengths and estimates of substitution model parameters could be obtained. This tree, with ML branch lengths and estimates of ML model parameters, was then used with Seq-Gen (Rambaut and Grassly, 1997) to simulate 100 replicate data sets, each with the same number of nucleotide sites as the original data set (2,079). These simulated data sets were then subjected to constrained and unconstrained parsimony tree searches as done before on the original data set. Tree scores for both constrained and unconstrained trees were extracted from PAUP* log files with a C++ program. Score differences between constrained and unconstrained trees were then used to generate the null distribution to which the test statistic was compared for determinations of significance (Hillis et al., 1996b; Goldman et al., 2000).

Analysis of Speciation Rates

New methods have recently become available to study the tempo of speciation. For example, several recent studies have included so-called lineage-through-time plots to study speciation rates among lineages (Nee et al., 1994; Barraclough et al., 1999; Lovette and Bermingham, 1999). This type of plot shows the number of lineages (on a log scale) as a function of time (Rambaut et al., 1997; Schluter, 2000; Barraclough and Nee, 2001). If speciation rates are constant through time then the plot is predicted to be linear, an expectation based on a pure-birth stochastic model (Barraclough and Nee, 2001). Plots that depart from this model by showing, say, upturns can be interpreted as having undergone a momentary increase in speciation rate or an illusory increase due to a decrease in extinction rate (Barraclough and Nee, 2001). We used the program End-Epi (Rambaut et al., 1997) to analyze speciation rates among lineages of pygopodid lizards in an effort to elucidate tempo of speciation in the group's history.

In a second analysis of speciation rates, we performed a rapid cladogenesis test, which is also implemented in End-Epi. In this analysis, a molecular phylogenetic tree under the molecular clock is input into End-Epi, which then calculates a cladogenesis statistic for each internal branch. This statistic, calculated for all nodes, represents the probability that lineage X occurring at time Y will split into Z number of species (at the tips) by the present time under a constant-rates birth-death model (Rambaut et al., 1997). Any clade with a P-value of <0.05 is considered to have undergone a higher than expected rate of speciation.

Calibration of Molecular Clock Trees

Fossil taxa can be used to calibrate molecular clock trees, thereby enabling investigators to estimate divergence times for the phylogeny of interest. The effectiveness of such a fossil for calibrating a molecular phylogeny is greatly enhanced when the fossil can be unambiguously inserted between two internal nodes, because then

upper and lower time bounds are established. Fortunately, such a fossil was available to us. *Pygopus hortulanus* is a recently described fossil pygopodid from the Riversleigh fossil beds in Australia (Hutchinson, 1997). These limestone deposits in northwest Queensland were formed 20–23 million years ago (MYA) during the early Miocene (Hutchinson, 1997). The fossilized remains consist of an intact and fully toothed dentary bone, which is very similar to but relatively plesiomorphic compared with the dentaries of extant *Pygopus lepidopodus* and *P. nigriceps.* However, the fossil animal is clearly distinguishable from *P. lepidopodus* and *P. nigriceps* in tooth structure and proportions of the symphysial region of the dentary (Hutchinson, 1997). Therefore, this fossil can be placed in the pygopodid phylogeny along the branch immediately below the node leading to *P. lepidopodus* and *P. nigriceps* but above the node leading to *P. orientalis*, which is the living sister species to *P. lepidopodus* and *P. nigriceps* (M. Hutchinson, pers. com., 1999; also see Results). Ranges of divergence times, rather than a single date, are provided for each speciation event because of uncertainty in the placement of the fossil calibration point along the internode and some imprecision associated with dating the age of the fossil.

Biogeographic Analysis

Phylogenetic information for a group of closely related species when merged with data on their present-day geographic ranges represents a powerful method for elucidating historical processes that drove a group's diversification. We therefore analyzed the historical biogeography of selected clades of pygopodids by examining present-day ranges of species in the context of their phylogenetic relatedness to closely related species. We constructed species range maps using data provided by Ehmann (1992), and phylogenetic information was obtained from the present study.

RESULTS

Data Characteristics

Following removal of all primer sequences and unalignable nucleotide sites, the mtDNA data set consisted of 1,706 bp, the nDNA set had 373 bp, and the combined data set contained 2,079 sites (Table 3). The entire *ND2* fragment could not be amplified for *D. mitella* or *D. pax* (probably because of degraded templates). However, a 738-bp portion of this fragment was successfully amplified and sequenced in both these taxa using primers ND2c and ND2f (Table 2). As expected, the mtDNA data seemed to be more variable than the nDNA data; nearly half of the sites in the former were variable but only 18% of the sites in the latter exhibited any variability (Table 3). Average base frequencies in the mtDNA set appeared to be unequal, with slight biases in A and C nucleotide frequencies, but those in the nDNA set were nearly equal (Table 3).

Analysis of each molecular data set using Modeltest suggested that the GTR + Γ + I model best fits the mtDNA data, the K80 + Γ model best fits the nDNA, and the GTR + Γ + I model best fits the combined molecular data set. Models and associated parameter estimates are presented in Appendix B on the *Systematic Biology* Web site.

Phylogeny Inferred from mtDNA Data

Maximum parsimony analyses of the mtDNA set produced two equally parsimonious trees (length [L] = 3884, consistency index [CI] = 0.37, retention index [RI] = 0.46, rescaled consistency index [RC] = 0.17, homoplasy index [HI] = 0.63), both of which were topologically similar to the ML and Bayesian trees (Fig. 2). Bootstrap proportions (BPs) in the shallow portions of these trees were generally high (≥50%), whereas deeper nodes were less well supported (Fig. 2). In each tree, the outgroup branch was connected to *D. concinna*, thereby rendering *Delma* paraphyletic. However, lack of monophyly for *Delma* may simply be due to improper root placement, a theme we reexamine later. Indeed, this root position is not well supported in either parsimony or likelihood trees (BPs of 29% and 32%, respectively; Fig. 2c).

An unanticipated finding, however, concerns the apparent paraphyly of *D. fraseri* in each tree (Fig. 2). Although the sister group relationship between *D.f. fraseri*/*D. grayii* and *D. f. petersoni* has low bootstrap support in the parsimony and likelihood trees (BP = 47%), the large BP (80%–87%) for the *D. f. fraseri*/*D. grayii* group suggests that the hypothesis of monophyly of *D. fraseri* should be rejected. Bayesian results support this finding more strongly. Parametric bootstrapping soundly

TABLE 3. Descriptive statistics for separate and combined data phylogenetically analyzed.

Data	16S	*ND2*[a]	*c-mos*	16S + *ND2*	16S + *ND2* + *c-mos*
Final length (bp)[b]	515	1191	373	1706	2079
No. variable sites (%)	168 (33)	695 (58)	69 (18)	863 (51)	932 (45)
No. parsimony-informative sites (%)	101 (20)	556 (47)	37 (10)	657 (39)	694 (33)
Average nucleotide frequencies					
A	0.38	0.33	0.27	0.35	0.33
C	0.23	0.32	0.21	0.29	0.28
G	0.18	0.11	0.24	0.13	0.15
T	0.21	0.24	0.28	0.23	0.24

[a] Includes 258 bp of flanking tRNA sequences.
[b] After removal of primer sequences and ambiguous alignment sites.

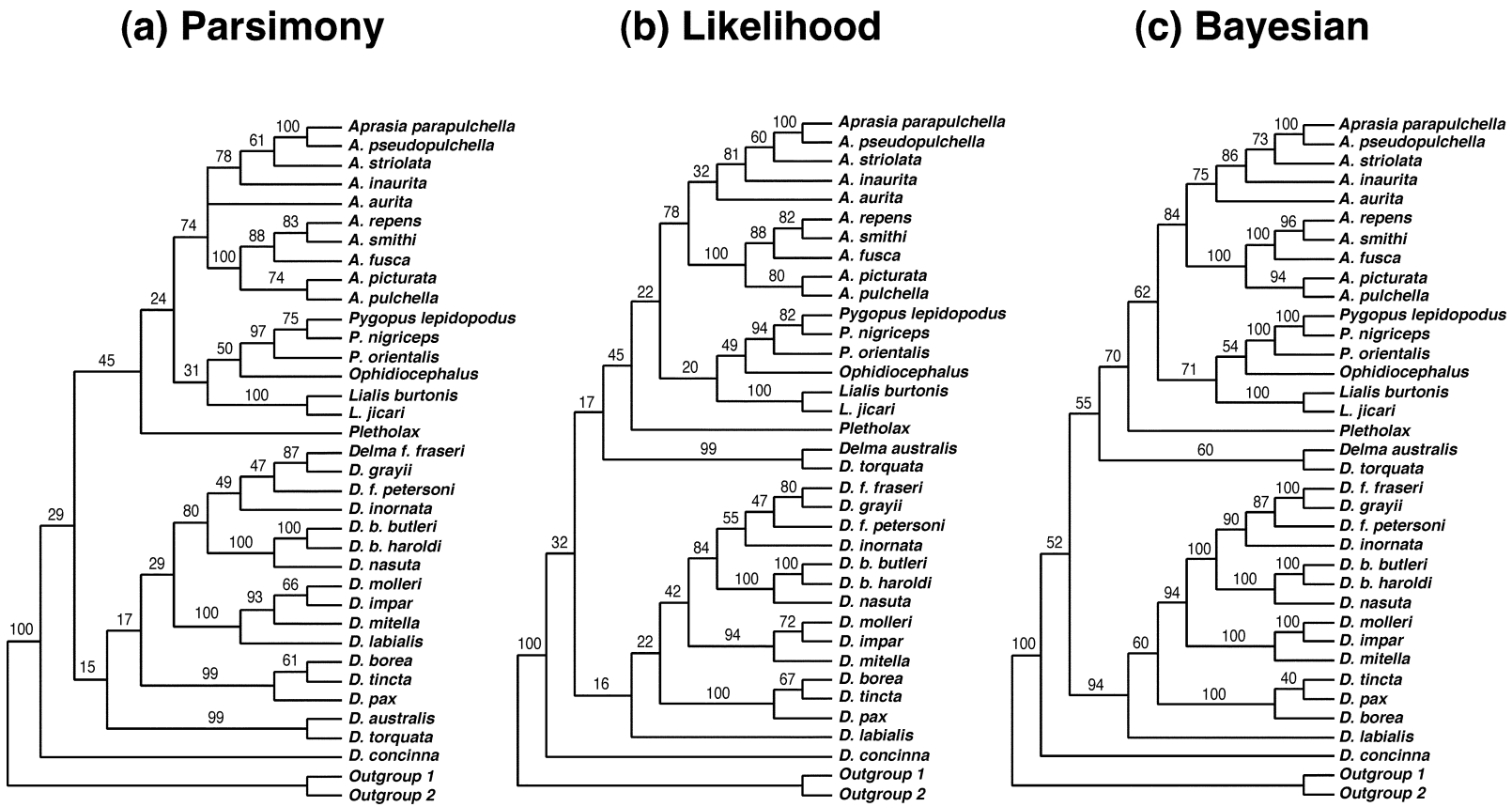

FIGURE 2. Phylogenetic hypotheses for 34 pygopodid taxa based on the mtDNA data set. (a) Strict consensus of two most-parsimonious trees; (b) ML tree based on the GTR + Γ + I substitution model; (c) Bayesian tree based on the GTR + Γ + I substitution model. Trees were rooted by two outgroup taxa (*Diplodactylus damaeus* and *D. tessellatus*). Numbers adjacent to each branch on parsimony and ML trees are bootstrap proportions, whereas numbers on the Bayesian tree are posterior probability values.

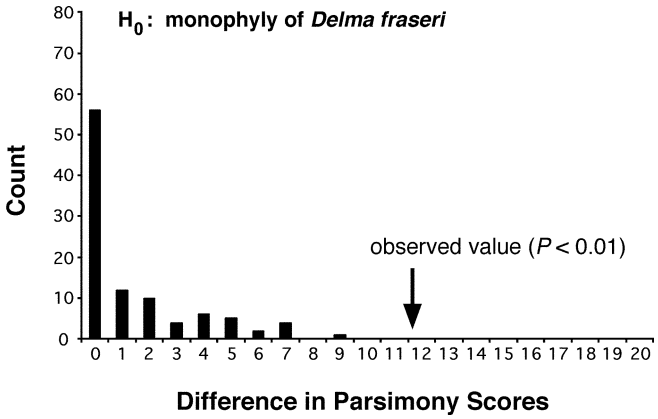

FIGURE 3. Results of parametric bootstrapping. Test of null hypothesis (H_0) that *Delma fraseri fraseri* and *D. f. petersoni* are monophyletic. Arrow indicates observed difference in parsimony scores.

rejects monophyly of *D. fraseri* (Fig. 3). We also eliminated the possibility that these results were due to invalid tissue samples, lab mix-ups, etc., by obtaining identical sequences from tissue samples taken from other individuals (one per taxon; Table 1).

Phylogeny Inferred from nDNA Data

A single most-parsimonious tree resulted from a parsimony analysis of the nDNA set (L = 92, CI = 0.83, RI = 0.92, RC = 0.76, HI = 0.17; Fig. 4a). The relatively lower HI for the nDNA tree suggests that the nuclear sequences are more conserved than the mitochondrial sequences. Phylogenetic analysis of these data using parsimony, ML, and Bayesian methods resulted in trees with identical topologies (Fig. 4). The conservative nature of *c-mos* was evident in that it did not resolve relatively shallow parts of the tree, whereas several deeper areas of the tree were well resolved. For example, *c-mos* provides reliable evidence for the monophyly of *Aprasia*, *Delma*, and *Pygopus* (Fig. 4). Monophyletic *Aprasia* and *Pygopus* were also observed in the mtDNA trees (Fig. 2). Although our mtDNA was able to confidently recover a monophyletic *Lialis* (Fig. 2) consistent with Kluge's (1976) analysis, the nDNA was unable to group these two taxa (Fig. 4). Another interesting feature of these three trees is that, in contrast to the mtDNA trees (Fig. 2), the root is connected to the *Lialis burtonis* branch (Fig. 4), raising the number of hypothesized root locations to three.

Our *c-mos* sequences did resolve some relationships deep within *Delma*, which contrasts with the mtDNA results (Figs. 2, 4). In particular, the three most-basal nodes in *Delma* and a novel *D. concinna*/*D. labialis* clade all received high bootstrap support (Fig. 4). These results were obtained regardless of whether trees were generated using parsimony, ML, or Bayesian methods. Thus, in contrast to mtDNA results, *c-mos* was better for resolving the placement of *D. concinna* and *D. labialis* in the tree (Figs. 2, 4).

Phylogeny Inferred from a Combination of mtDNA + nDNA Data

A single most-parsimonious tree resulted from a parsimony analysis of the combined mtDNA + nDNA set (L = 3987, CI = 0.38, RI = 0.48, RC = 0.18, HI = 0.62; Fig. 5a). Analysis of these data using ML and Bayesian methods resulted in two trees with identical ingroup relationships (Figs. 5b, 5c). However, the parsimony tree differs from the likelihood and Bayesian trees in its placement of *Pletholax*, *Delma labialis*/*D. concinna*, and the root (Figs. 5a–c). Like all previous parsimony and ML trees, bootstrap support for the deepest branches in the tree remains weak.

Combining data caused fluxes in levels of clade support. For example, combining molecular data sets increased support for *Aprasia* monophyly (BP of 74%–81% vs. 86%–88%), whereas support for the deepest nodes in *Delma* and for *D. concinna*/*D. labialis* monophyly decreased. However, strong posterior probability support for *Aprasia* monophyly was obtained in every Bayesian analysis (Figs. 2c, 4c, 5c). Combining data did not affect the majority of nodes where adequate bootstrap support had been originally found in separate data analyses. Unfortunately, combining data did not help resolve intergeneric relationships, although the fairly consistent placement of *Ophidiocephalus* as sister to *Pygopus* may be an exception.

Phylogeny Inferred from a Combination of Morphological and Molecular Data

Parsimony analysis of the combined morphological and molecular data yielded six equally parsimonious trees (L = 4248, CI = 0.39, RI = 0.51, RC = 0.20, HI = 0.62; Fig. 6a). Most intrageneric relationships in this strict consensus tree match those found in the molecular trees, whereas most of the intergeneric groupings reflect Kluge's tree. The one intergeneric grouping that is not consistent with Kluge's tree is the *Delma*/*Lialis* clade, which was only previously found in all nDNA trees (compare Figs. 1, 4, and 6a). Another feature of this tree that is not consistent with Kluge's phylogenetic hypothesis is the placement of the root along the *Pletholax* branch (compare Figs. 1 and 6a). Bayesian analysis of the combined morphological and molecular data generated a tree with almost the same ingroup topology and level of clade support as found in the parsimony tree (Fig. 6b). One major difference between trees is that *Lialis* and *Pygopus* switched places, thereby creating a *Delma*/*Pygopus* clade in the Bayesian tree rather than a *Delma*/*Lialis* clade, which was found in the parsimony tree (Figs. 6a, 6b). A second difference is that the outgroup is attached to *Lialis* in the Bayesian tree, whereas it rooted the parsimony tree along the *Pletholax* branch (Figs. 6a, 6b).

The preceding results raise two troublesome issues concerning pygopodid phylogeny estimation. First, ingroup relationships above the generic level showed little consistency among trees. This variability was observed among trees derived from different data sets

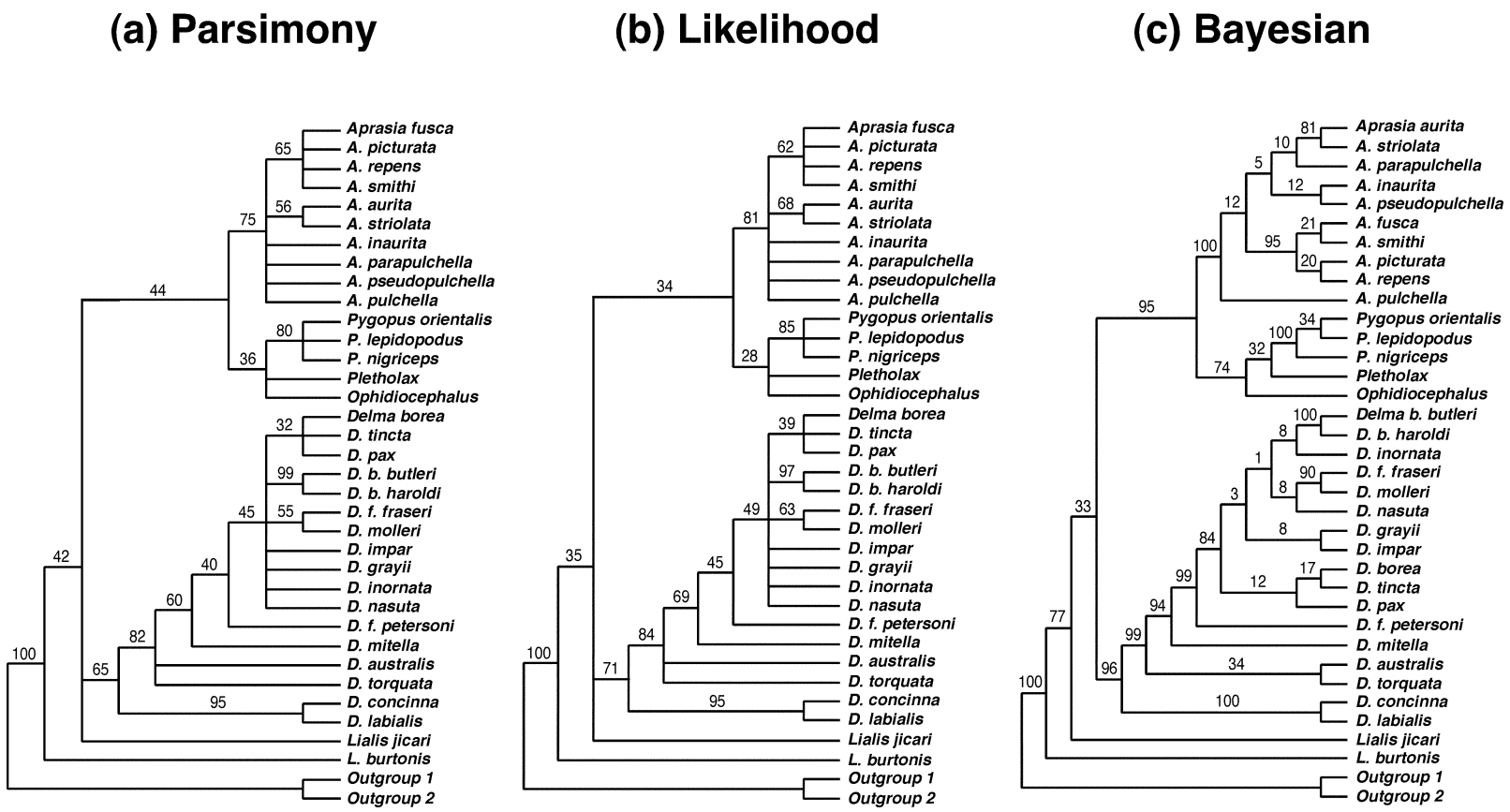

FIGURE 4. Phylogenetic hypotheses for 34 pygopodid taxa based on the nDNA data set. (a) Single most-parsimonious tree resulting from parsimony analysis; (b) ML tree based on the K80 + Γ substitution model; (c) Bayesian tree based on the K80 + Γ substitution model. Trees were rooted by two outgroup taxa (*Diplodactylus damaeus* and *D. tessellatus*). Numbers adjacent to each branch on parsimony and ML trees are bootstrap proportions, whereas numbers on the Bayesian tree are posterior probability values.

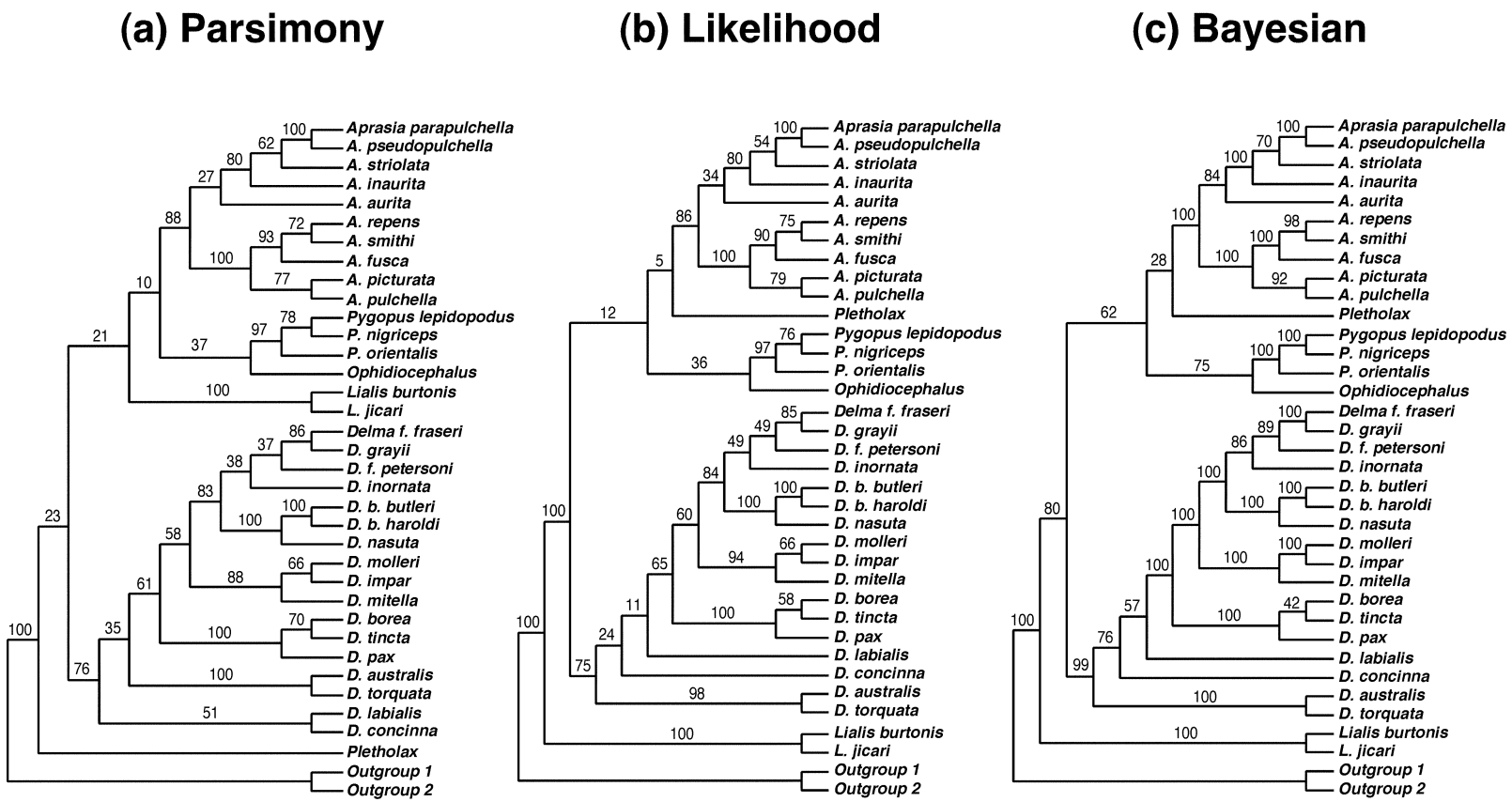

FIGURE 5. Phylogenetic hypotheses for 34 pygopodid taxa based on combined mtDNA + nDNA data. (a) Single most-parsimonious tree resulting from parsimony analysis; (b) ML tree based on the GTR + Γ + I substitution model; (c) Bayesian tree based on the GTR + Γ + I substitution model. Trees were rooted by two outgroup taxa (*Diplodactylus damaeus* and *D. tessellatus*). Numbers adjacent to each branch on parsimony and ML trees are bootstrap proportions, whereas numbers on the Bayesian tree are posterior probability values.

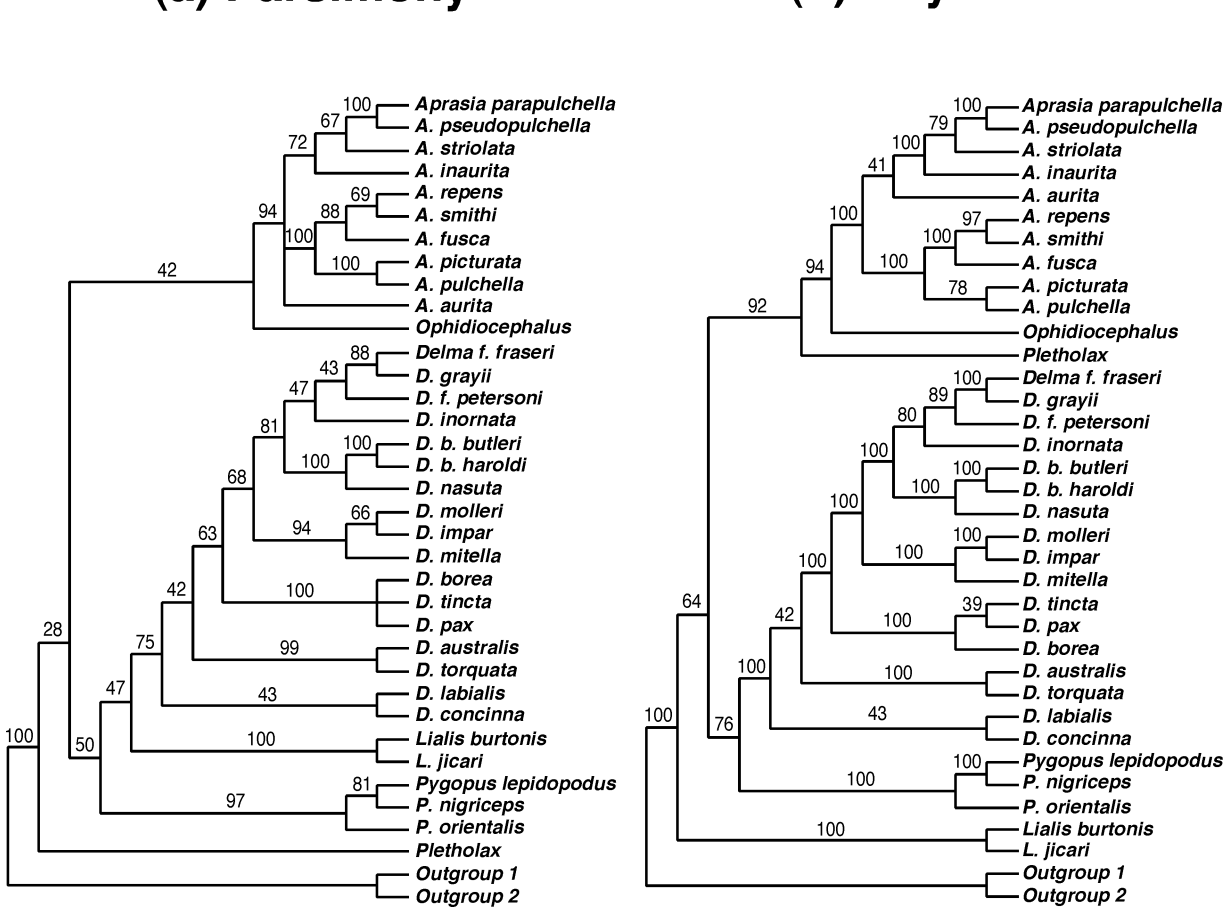

FIGURE 6. Combined morphology and molecular (mtDNA + nDNA) trees for 34 pygopodid taxa. (a) Strict consensus tree of six most-parsimonious trees; (b) Bayesian tree based on the GTR + Γ + I substitution model. Trees were rooted by two outgroup taxa (*Diplodactylus damaeus* and *D. tessellatus*). Numbers above each branch in the parsimony tree are bootstrap proportions, whereas numbers on the Bayesian tree are posterior probability values.

(Figs. 2, 4–6), and even among trees generated from a single data set but using different optimality criteria (e.g., Fig. 5). The second issue concerns the proper location of the root. Outgroup rooting of all trees suggests no less than three different rooting locations on the pygopodid phylogeny. Moreover, Kluge (1976) suggested that the root should be attached to *Pygopus lepidopodus*. Inclusion of a highly divergent outgroup may not only confuse the rooting issue but may actually interfere with the reconstruction of ingroup relationships (Swofford et al., 1996). Some of our results suggest that rooting the ingroup using outgroups may be fruitless. For example, ML analysis of combined mtDNA + nDNA data with both outgroup taxa included produces a different ingroup topology than if the outgroups are excluded (Fig. 5).

Phylogenetic Analysis of the Pygopodids, Outgroups Excluded

In an attempt to improve resolution of ingroup relationships, we performed all the same phylogenetic analyses as before except that both outgroup taxa were excluded. Support for intergeneric relationships in the Bayesian trees varied substantially (Fig. 7). For example, whereas *Delma/Lialis* monophyly in the parsimony and ML nDNA trees received poor support (BP = 43%–44%), this arrangement was strongly supported in the Bayesian tree (posterior probability = 98; Fig. 7). In contrast to outgroup-included analyses, estimation of ingroup relationships based on each of the molecular data sets was not sensitive to method of tree optimization (Fig. 7). However, like the outgroup analyses, ingroup relationships differed dramatically among data sets, particularly between molecular and morphological data (Fig. 7). Within the set of trees based on molecular data, the primary difference seems to be the placement of *Pletholax* and *Lialis* (Fig. 7). In the mtDNA and combined mtDNA + nDNA trees, *Delma* and *Pletholax* form a group, whereas in the nDNA trees *Delma* and *Lialis* are sister to each other (Fig. 7). Neither arrangement is strongly supported in parsimony and ML trees, but the *Delma/Lialis* grouping is well supported in the Bayesian

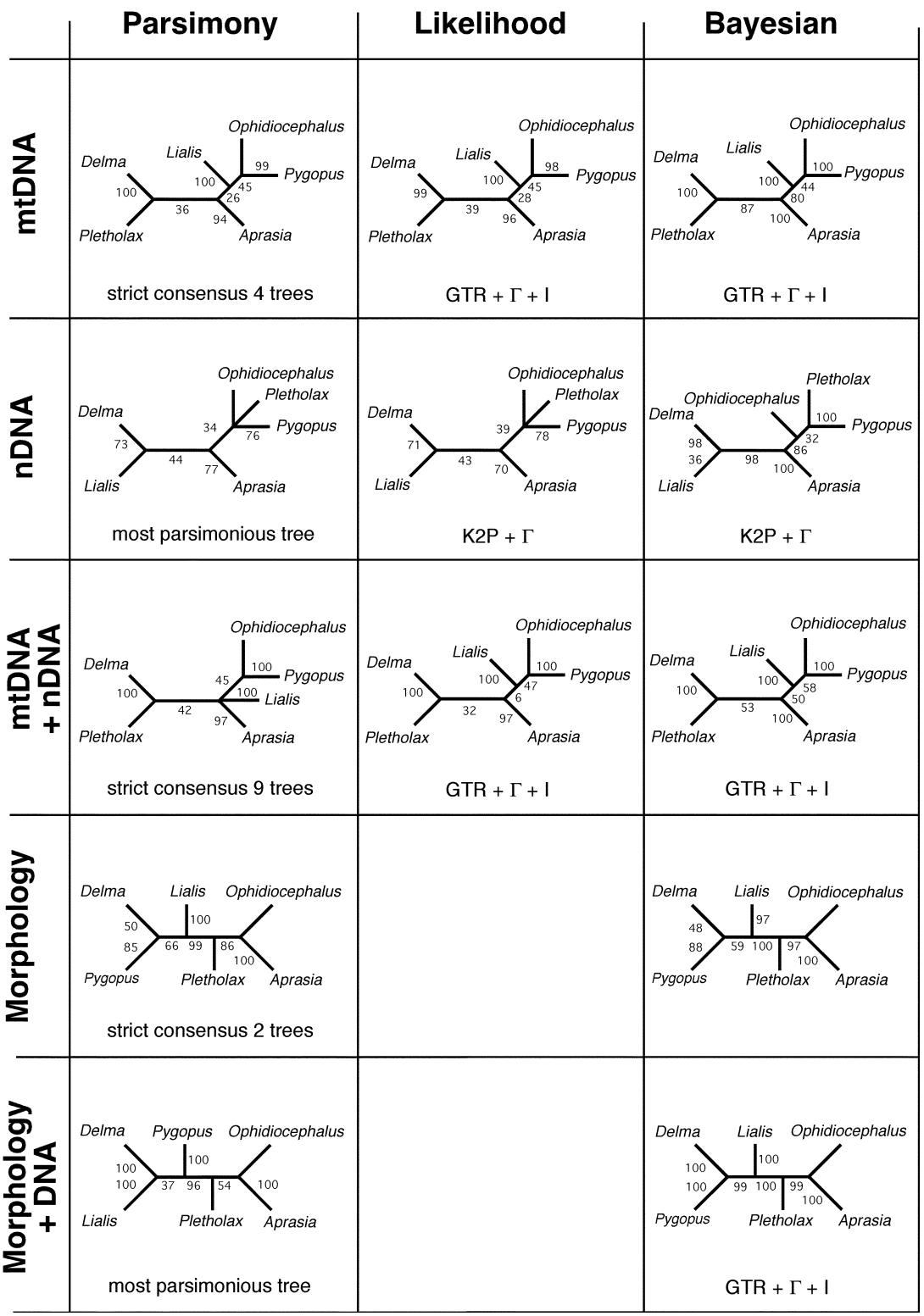

FIGURE 7. Unrooted trees showing inferred relationships among six well-supported ingroup lineages based on mtDNA, nDNA, mtDNA + nDNA, morphology, and morphology + DNA data sets. Phylogenetic analyses of each data set were performed in a manner similar to those that created the trees in Figures 2, 4, 5, and 6, except that outgroup taxa were omitted. Numbers above each branch on parsimony and ML trees are bootstrap proportions, whereas numbers on Bayesian trees are posterior probability values.

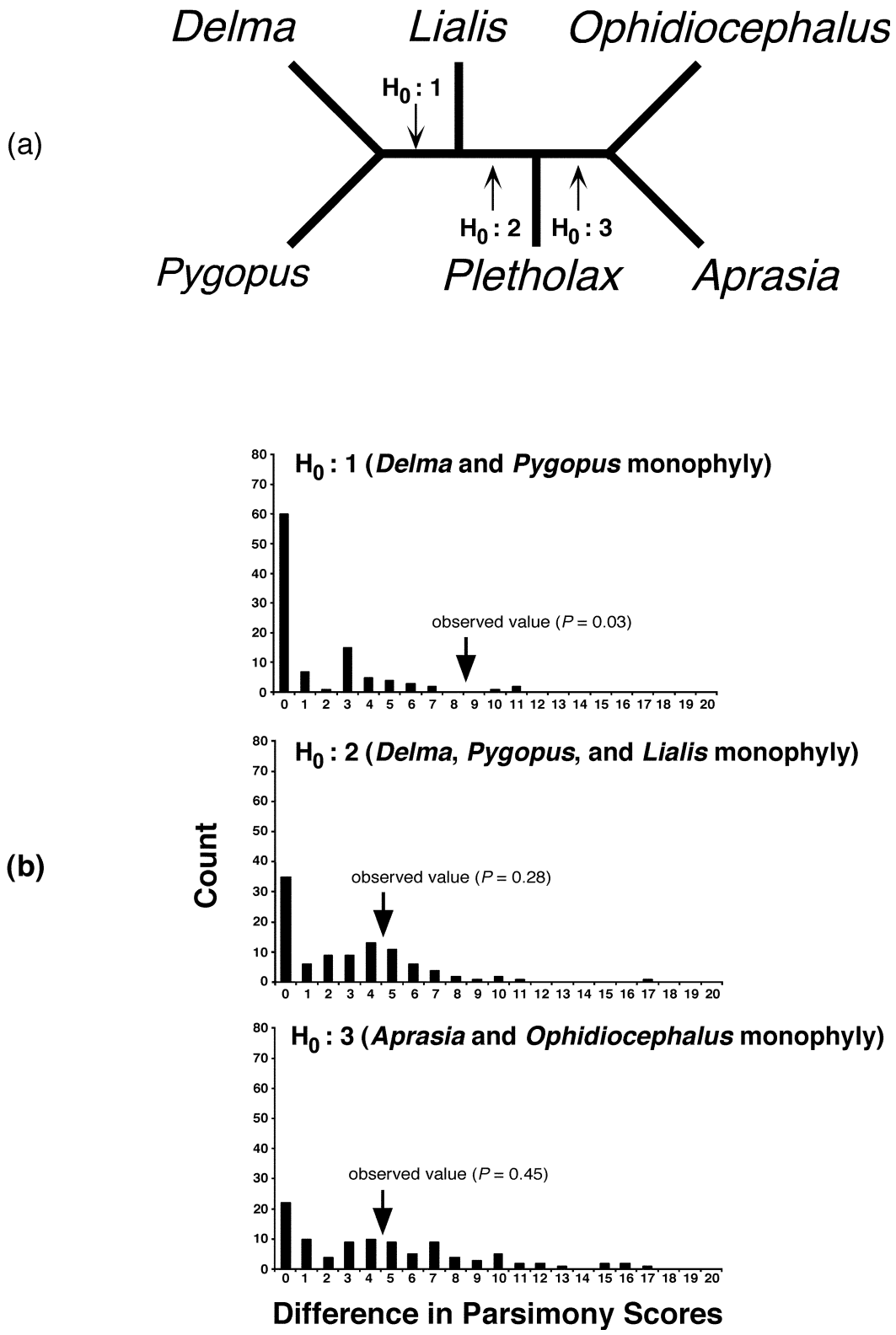

FIGURE 8. (a) Unrooted morphology tree (from Fig. 7) depicting three null hypotheses (H_0) concerning the branching relationships among six pygopodid lineages. (b) Results of parametric bootstrap analyses. Arrows indicate observed difference in parsimony scores.

nDNA tree (Fig. 7). In the morphology tree, two of the three internal branches are well supported (BP = 99% and 86%), which contrasts with all the parsimony molecular trees (Fig. 7). When molecular and morphological data are combined and analyzed using parsimony, the resulting tree mostly resembles the morphology tree except that the positions of *Lialis* and *Pygopus* are reversed, reflecting the arrangement found in the nDNA trees. The topology of the Bayesian tree actually matches that of the morphology tree, plus its three internal branches have strong support (Fig. 7).

Unfortunately, exclusion of outgroup taxa from phylogenetic analyses of each data set did not lead to unambiguous resolution of the three branches connecting the six generic lineages. Branching relationships differed most dramatically between molecular and morphological trees, perhaps because the molecular data set is insufficient to completely resolve the tree or the morphological data set is riddled with homoplastic characters.

Aprasia, *Delma*, *Lialis*, *Ophidiocephalus*, *Pletholax*, and *Pygopus* represent the largest unambiguously supported inclusive clades. The problem of finding the correct three branches that link these six lineages can be addressed through hypothesis testing in a statistical framework. Each of the three internal branches in the morphology tree (from Fig. 7) can be considered a null hypothesis (Fig. 8a), which can be statistically evaluated using parametric bootstrapping. Parametric bootstrapping analyses indicate that the first null hypothesis (*Delma* and *Pygopus* monophyly) can be rejected by the combined molecular data ($P = 0.03$; Fig. 8b). However, the second (*Delma*, *Pygopus*, and *Lialis* monophyly) and third (*Aprasia* and *Ophidiocephalus* monophyly) null hypotheses could not be rejected ($P = 0.28$ and $P = 0.45$, respectively; Fig. 8b), suggesting that either the combined molecular data set is simply insufficient to reject these hypotheses (type II statistical error) or that the null hypotheses are correct. Given these results, our preferred unrooted tree is the parsimony morphology + DNA tree (Fig. 7) because it has *Delma* and *Lialis* as a clade but retains the other two branches found in the morphology tree.

Inferring the Root of the Pygopodid Tree Using the Molecular Clock Method

As pointed out earlier, the diplodactyline outgroup rooted the pygopodid tree in three different places depending on data set and method of analysis, indicating that it performed poorly in rooting the pygopodid tree. The root positions suggested by various analyses are summarized in Figure 9, and their frequencies of occurrence are listed in Table 4. The mtDNA data did not meet the clock assumption ($\delta = 110$, $\chi^2_{[0.05]} = 46$, $P < 0.05$), whereas the nDNA did ($\delta = 39$, $\chi^2_{[0.05]} = 46$, $P > 0.1$). However, both data sets rooted the pygopodid tree at the same location (e.g., location 5; Fig. 9; Table 4). The branch leading to *Lialis* (root location 3), which is adjacent to root location 5, was found in six outgroup-rooted trees (Table 4). We favor the clock-root estimate over any of the outgroup roots simply because the chance of two independent data sets converging upon the same branch is quite unlikely (~1 in 65 odds). Figure 10 shows our revised pygopodid tree reflecting our preferred ingroup topology and root position.

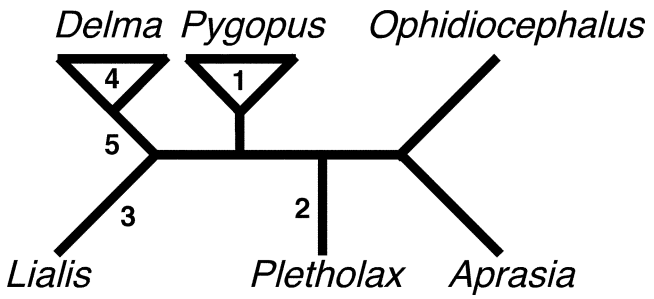

FIGURE 9. Most well-supported phylogenetic hypothesis of pygopodid ingroup relationships illustrating five different rooting locations as suggested by various phylogenetic hypotheses. Location 1 was suggested by Kluge (1976), whereas all other root positions were obtained from outgroup and molecular clock rooted trees in this study.

Speciation Rates

The plot of lineages through time (Fig. 11) mostly exhibits an apparently linear trend except for two pronounced upturns and one long downturn. The first upturn, which occurred between 23 and 33 MYA, is explained by speciation events at nodes B, R, and S, whereas the second upturn, which happened some time between 17 and 23 MYA, is explained by lineage splitting events at nodes I, U, and W (Fig. 11). In addition to these upturns, a dramatic slowdown in the speciation rate also seems to have occurred over the last 10 million years (Fig. 11). The final observation gleaned from this analysis is that, although the most recent common ancestor to extant pygopodids existed approximately 37 mya, 28 of 33 extant species included in our study originated in the last 23 million years.

Relative cladogenesis tests suggest that clades W, D, and F may have rapidly diversified, but these results were not statistically significant ($P = 0.078$, 0.056, and 0.056, respectively). However, these P-values would probably be significant had we included the two additional species of *Aprasia* and two of *Delma* missing from our study because the latter two P-values actually do become significant if the additional subspecies of *D. butleri*

TABLE 4. Frequency of occurrence for various root locations on the pygopodid tree based on outgroup and molecular clock rooting analyses. Numbers in table refer to specific root locations (see Fig. 9), and asterisks indicate cases in which the root could not be estimated.

	Outgroup			Molecular clock
Data set	Parsimony	ML	Bayesian	
mtDNA	4	4	4	5
nDNA	3	3	3	5
mtDNA + nDNA	2	3	3	5
morphology + DNA	2	*	3	*

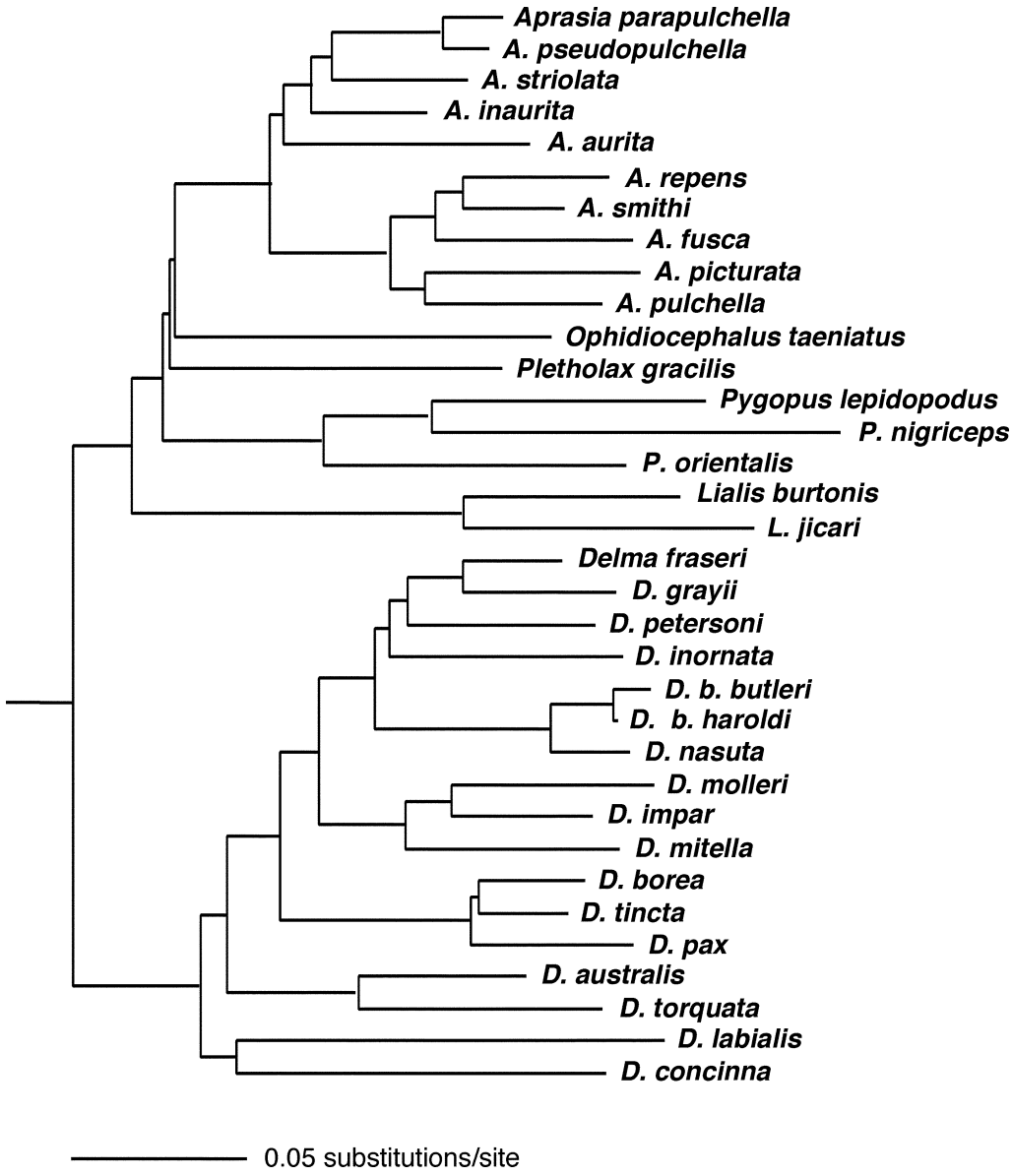

FIGURE 10. Revised phylogenetic hypothesis for pygopodid lizards: ML phylogram with ingroup relationships suggested by analysis of combined morphology + DNA data. Branch lengths were estimated via ML using a GTR + Γ + I substitution model. Root location reflects results of clock-rooting analyses.

is included. All other clades had probabilities ranging from 0.22 to 1.0.

Biogeographic Patterns

Clade W exhibits some geographic structure and reveals a previously unknown deep divergence that resulted in the formation of western and eastern geographic clades (Fig. 12). Interestingly, populations of *A. striolata* are found in South Australia and adjacent islands, in possibly in the Northern Territory, and in Western Australia (Fig. 12). The overall distributional pattern exhibited by Clade W can be characterized as a group of highly fragmented and small populations restricted to the mesic-temperate zones of Australia. Indeed, the present distribution of this group correlates to a strikingly high degree with the areas that have high winter rainfall regimes (i.e., Mediterranean climate zone). Clades E, P, and C (Fig. 11) reveal a pattern in which sister species are geographically widely spaced from each other (Figs. 13a–c). In particular, the spatial distance found between *D. concinna*, which is confined to the west coast, and *D. labialis* on the east coast is staggering (Fig. 13c). Curiously, *D. labialis* and *D. mitella* seem to exist as tiny isolates in the wet tropics of northeast Queensland (Figs. 13b, 13c). The remaining clades G, K, and L (Fig. 11), show a different pattern, one in which sister species are found in broad sympatry (Figs. 13d–f). Moreover, these sympatric sister species are largely found in arid and semiarid parts of Australia (Figs. 13d–f).

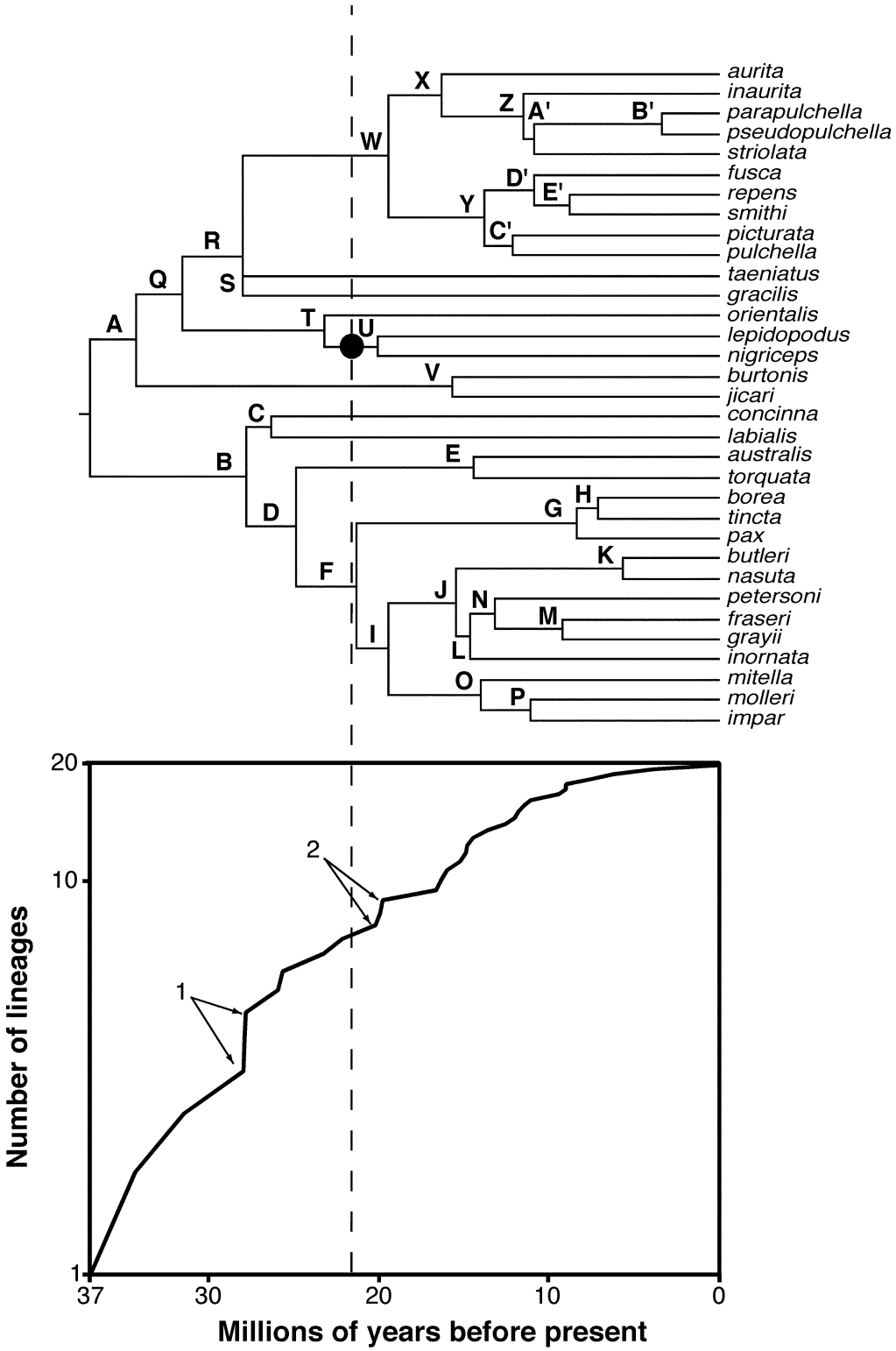

FIGURE 11. Pygopodid molecular clock tree (top) based on combined mtDNA and nDNA data and a plot of lineages through time (bottom). All nodes are labeled with letters as referred to in the text. Solid dot on the branch between nodes T and U is the fossil calibration point, which corresponds to approximately 20–23 MYA. Numbers 1 and 2 on the semilog plot indicate sections of each line that may have undergone increases in speciation rates and correspond to time periods of 23–33 MYA and 17–23 MYA, respectively (after Schluter, 2000; Barraclough and Nee, 2001).

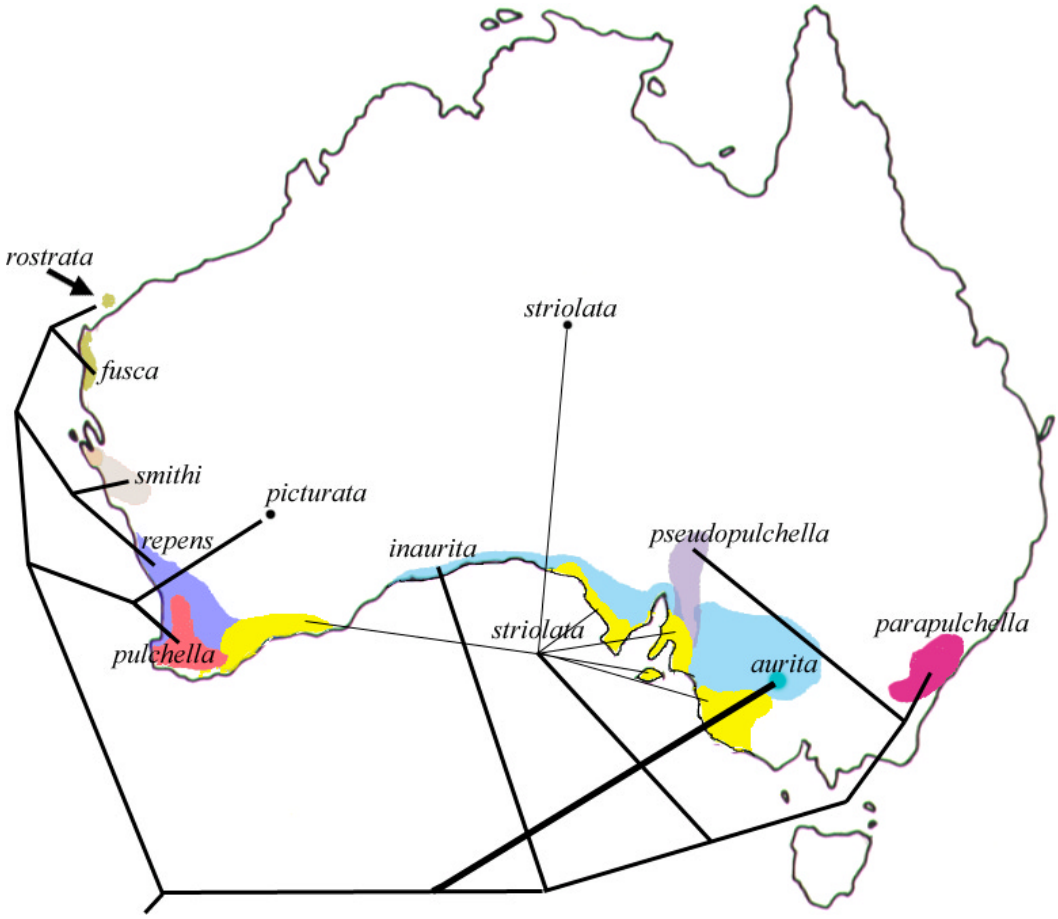

FIGURE 12. Inferred historical relationships among species in *Aprasia* (clade W in Fig. 11) in the context of present geographic distributions. Individual species' ranges are distinguished by colors (after Ehmann, 1992). Species tree was taken from Figure 10. Intraspecific relationships among populations of *A. striolata* were not estimated, so a polytomous branching scheme is shown.

DISCUSSION

Phylogeny of the Pygopodidae

Based on morphological data, Kluge (1976:67) concluded that *Aprasia*, *Delma*, *Lialis*, *Ophidiocephalus*, *Pletholax*, and *Pygopus* represented the only well-supported clades in his phylogenetic tree. Results of our analyses of mtDNA and nDNA data, regardless of whether parsimony, ML, or Bayesian tree optimization methods were used, strongly support existence of these taxa plus provide for the first time robustly supported intrageneric relationships. The branching structure among these six genera, however, proved more problematic; little agreement was found among mtDNA, nDNA, and morphological trees. More troubling, our outgroup taxa are so molecularly divergent from pygopodids that their inclusion interfered with the estimation of ingroup relationships and rendered the root location ambiguous. We therefore followed the advice of Swofford et al. (1996) and reanalyzed our data without outgroups and attempted to resolve the rooting issue by means other than the traditional outgroup method.

The question of combining different data sets in a phylogenetic analysis has been controversial (see Bull et al., 1993; de Queiroz, 1993). Aside from any putative pros and cons of combining data, the popular incongruence length difference test (Farris et al., 1994) as a test of data set combinability (Cunningham, 1997a, 1997b; Swofford, 2000) has recently been criticized on statistical grounds (Barker and Lutzoni, 2002; Darlu and Lecointre, 2002). Although we carefully examined our results based on each data set alone—each one after all represents an independent estimate of phylogeny—we opted for combining our data in hopes of improving overall phylogenetic signal. Additional justification comes from the observation that the morphological and molecular trees shared several clades, demonstrating that at least some concordant phylogenetic signal exists among data sets, and from the fact that this approach has been the norm in systematics (Hillis, 1987; Olmstead and Sweere, 1994; Hillis et al., 1996b).

Combined mtDNA and nDNA analyses yielded trees that largely retained the relationships found only in the mtDNA tree but not in the nDNA tree, particularly among congeners. This result is not surprising given that the mtDNA data set contained 657 parsimony-informative characters as opposed to a mere 37 in the nDNA data. The mtDNA data also had much higher

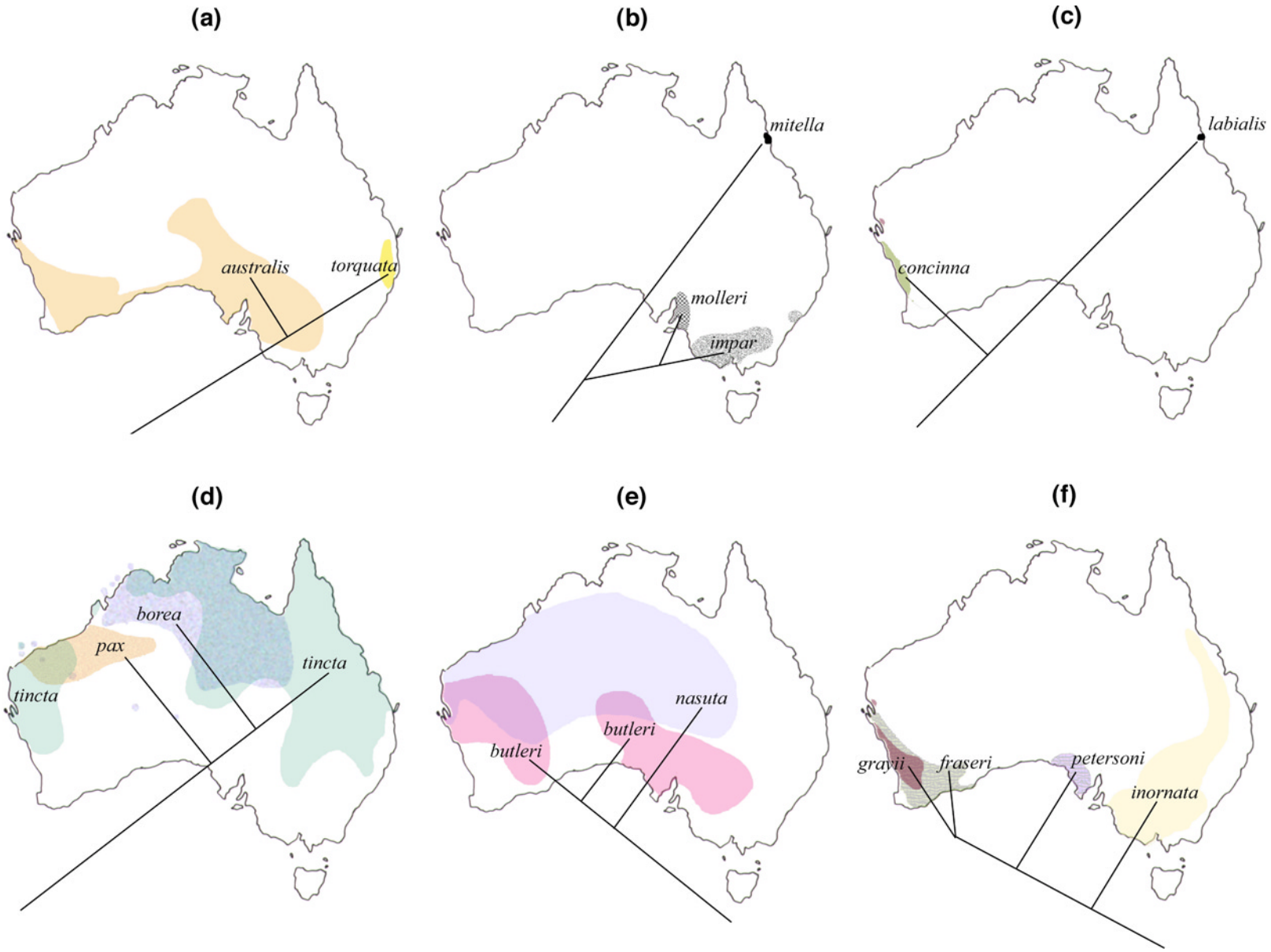

FIGURE 13. Inferred historical relationships among species of *Delma* in clades E (a), P (b), C (c), G (d), K (e) and L (f), in the context of their present geographic distributions. Individual species' ranges are distinguished by colors (after Ehmann, 1992). Clades are from the species tree in Figure 11.

levels of homoplasy (HI = 0.63 vs. 0.17, respectively), which could hinder efforts to recover deeper nodes in the tree. Indeed, branch support was very low in basal parts of *Aprasia* and *Delma* clades and in branches connecting generic lineages in the mtDNA trees and combined DNA trees. Reconstructing such deep level branching structure in vertebrate phylogenies using DNA sequence data seems to be a chronic problem (e.g., Kraus and Miyamoto, 1991; Shaffer et al., 1997; Jackman et al., 1999; Yuri and Mindell, 2002). However, we did succeed in creating a tree with well-supported branching structure throughout by combining molecular and morphological data. In parsimony and Bayesian trees, the intrageneric relationships mirrored the mtDNA results, whereas the intergeneric branching scheme largely reflected morphological trees, especially the Bayesian tree. The strongly supported intergeneric scheme in our Bayesian molecular and morphological tree on the one hand helps provide a well-resolved phylogenetic hypothesis for pygopodids, but on the other hand may be incorrect owing to strong levels of homoplasy in the morphological data. Moreover, our molecular data may suffer from low power and therefore could have been overwhelmed by the signal provided by morphological characters. Our parametric bootstrapping results rejected one of the three internal branches (null hypotheses), suggesting that the *Delma/Pygopus* clade present in the morphology tree may be incorrect. We therefore regard the parsimony morphology + DNA tree in Figure 7 the preferred topology because its alternative arrangement of *Delma/Lialis* monophyly and retention of the two other internal branches of the morphology tree (H_o: 2 and H_o: 3, Fig. 8) is compatible with our parametric bootstrapping results.

Rooting a tree with an outgroup that is too phylogenetically distant from the ingroup can result in a spurious root estimate because the outgroup sequences, which are effectively scrambled with respect to the ingroup sequences, may consequently be "attracted" to a long ingroup branch that is not necessarily the true root location (Hendy and Penny, 1989; Miyamoto and Boyle, 1989; Wheeler, 1990; Maddison et al., 1992; Swofford et al., 1996; Holland et al., 2003). This so-called long-branch attraction problem was so severe that our outgroup specified three different root locations in the pygopodid tree, depending upon the analysis, and hampered our ingroup estimation efforts. The question of the correct root location in the pygopodid tree is confused further when Kluge's (1976) hypothetical root is also considered, which differed from our outgroup estimates. We therefore abandoned the outgroup method and attempted to root our tree using a molecular clock. Application of this method to these data sets yielded consistent results; the same root location was suggested in all three analyses (i.e., position 5, Fig. 9). Because the root could connect into any of the 65 branches on the pygopodid tree, it is extremely unlikely that the same root location could be found by two independent data sets (mtDNA and nDNA) from chance alone (~1 in 65 odds). Although this particular root location, which is located on the branch leading to the *Delma* clade, was not found in any of the outgroup-rooted trees, it is certainly in the neighborhood of the majority of outgroup-selected locations: Three locations were located in a basal section of the *Delma* clade and six were found along the branch leading to *Lialis* (Fig. 9). Although the mtDNA data did not meet the clock assumption, the clock-root position suggested by these data did match the root location of the clock-obeying nDNA data set. Moreover, simulation results of Huelsenbeck et al. (2002) are encouraging because they suggest that a molecular data set can still find the correct root even when the sequences did not evolve in a strict clocklike manner.

Taxonomic Implications

Eight pygopodid genera were recognized in Kluge's (1974) taxonomic monograph on Pygopodidae. However, Kluge subsequently lowered this number to six following his cladistic study of the group, because two of the four monotypic genera, *Aclys* and *Paradelma*, appeared to be closely allied with *Delma* and *Pygopus*, respectively (Kluge, 1976). Despite this finding, few workers have adopted Kluge's new arrangements for *Aclys* (Shea, 1987b) and *Paradelma* (Wilson and Knowles, 1988; Greer, 1989; Cogger, 1992; Ehmann, 1992). Some, however, have directly or indirectly questioned the continued acceptance of *Aclys* (Greer, 1989) and *Paradelma* (Czechura and Covacevich, 1985; Shea, 1987a).

In all trees except the mtDNA trees, *Aclys* is either the sister species to *D. labialis* or is embedded elsewhere within the *Delma* clade (Figs. 4–6). With the exception of the combined molecular ML tree (Fig. 5b), most other trees suggest that *Aclys* is a member of the most basal lineage in *Delma*. *Paradelma* was found to be the sister taxon to a *Pygopus* clade, which differs slightly from Kluge's (1976) paraphyletic arrangement of *Pygopus*. In Kluge's (1976) analysis, *Paradelma* and *Pygopus nigriceps* formed a clade, whereas our reanalysis of his morphological data showed that *P. nigriceps* and *P. lepidopodus* actually form a well-supported clade, which mirrors the mtDNA results (Fig. 2). These molecular results are consistent with Kluge's six-genus scheme. Given the present uncertainty of the relationship of *Aclys* to *Delma*, reelevating *Aclys* to generic status is unwarranted—note that to do so would also necessitate reassigning *D. labialis* to the genus *Aclys* as well. Results concerning *Pygopus*, however, would fit with the recognition of *Paradelma*.

All but two described species of *Delma* were included in this study. The only species of *Delma* missing were *D. elegans* and *D. plebeia*. Because phylogenetic relationships among many of these species are being proposed for the first time, few comparisons with previous work are possible. The relatively derived positions occupied by *D. concinna* and *D. australis* in Kluge's (1976) *Delma* clade contrast with the more basal placement of these taxa in our trees. Both studies, however, do show a *D. molleri* + *D. impar* clade, a result that conflicts with Shea's (1991) *D. impar* group, which included *D. impar*, *D. plebeia*, and *D. torquata*. Our results suggest that *D. torquata*

is more closely related to *D. australis* than to *D. impar*. Our results also lend strong support to Shea's (1991) *D. tincta* group, which consists of *D. tincta*, *D. borea*, and *D. pax*. Shea justified this grouping because these forms share similar color patterns, largely replace each other geographically, and are diagnosable by the usual presence of only a single elongate upper temporal scale bordering the parietals, which is a derived feature among pygopodids. Based on geographic variation in morphology, Shea (1991) split *D. fraseri* into two subspecies: the nominate form *D. f. fraseri* endemic to southwestern Western Australia and a subspecific race, *D. f. petersoni*, known only from southeastern South Australia and adjacent parts of Western Australia. A surprising finding from this study suggests that *D. f. fraseri* is more closely related to the sympatric *D. grayii* than to the allopatric *D. f. petersoni*. Although results from our sampling of multiple individuals/taxon and parametric bootstrap analysis indicate we recovered the correct mtDNA gene tree, our tree may still differ from the population or species tree because of hybridization or incomplete lineage sorting (Moore, 1995; Avise, 2000). Hybridization seems unlikely given the 235-km distance between populations (Shea, 1991) coupled with the low vagility of these lizards. Incomplete lineage sorting, however, is a more plausible explanation for our results, so we will consider this idea further.

The topology of a three-species gene tree will match the species tree if the alleles or haplotypes have achieved reciprocal monophyly (i.e., complete lineage sorting) during the time interval between the first and second internal nodes (Hudson, 1992; Moore, 1995). Incomplete lineage sorting is therefore one major reason why using molecular data to recover the correct species tree for a group of species that rapidly radiated can be problematic even if the correct gene tree is reconstructed (e.g., African cichlids; Parker and Kornfield, 1997). Based on neutral coalescence theory, reciprocal monophyly of mtDNA haplotypes is expected to occur in $2N_{f(e)}$ generations, where $N_{f(e)}$ is the effective population size for females ($2N_{f(e)}$ is equal to $4N_{mt}$, where N_{mt} = effective number of mitochondrial gene copies in the population). Thus, the probability of having congruent gene and species trees is high when $T > 2N_{f(e)}$ generations and low when $T < 2N_{f(e)}$ generations (Hudson, 1992; Moore, 1995). By calibrating the molecular clock version of our mtDNA tree (not shown) with the 20–23 million year old pygopodid fossil (Hutchinson, 1997), we can estimate that the internal branch (internode) in the *D. fraseri* clade translates to a time interval of 3.6–4.9 million years. If we assume a generation time of 5 years for these lizards (a conservative guess), then $T = 720{,}000$–$980{,}000$ generations. For lineage sorting to still be incomplete within this time period, $N_{f(e)}$ must therefore exceed 490,000 females. This population size is already larger than historically possible for these lizards. The 3–5-million-year internode should therefore be more than adequate time for lineage sorting to occur prior to the gene divergence event giving rise to *D. grayii* and *D. f. fraseri* haplotypes. We are therefore inclined to accept that our gene tree does accurately depict the speciational history of these populations. Accordingly, we propose elevating *D. f. petersoni* to species status so that mtDNA paraphyly of *D. fraseri* is reconciled. This action is further supported by the fact that both subspecies are allopatric, are morphologically distinguishable from each other in terms of color pattern and scalation, and occupy different habitat niches (Shea, 1991).

Our study resolved nearly all relationships among 10 of the 12 species of *Aprasia*. Only *A. haroldi*, which is endemic to the Shark Bay region of Western Australia (Storr et al., 1990), and *A. rostrata* of Hermite Island near the northwestern coast of Western Australia (Kluge, 1974) were not included. Although no additional specimens of *A. rostrata* have been collected since its locality was subjected to atomic weapons testing in the 1950s (Ehmann, 1992), this species is probably most closely related to adjacent mainland populations of *A. fusca* (Storr et al., 1990). *Aprasia fusca* was originally described as a subspecies of *A. rostrata* by Storr (1979) but later was elevated to full species status by Storr et al. (1990). In these analyses, *A. fusca* formed a well-supported clade with *A. repens* and *A. smithi*, which supports Storr's (1979) allocation of *A. fusca* to the *A. repens* superspecies complex. Some agreement was found between Kluge's (1976) results and ours; both studies identified an ((*A. parapulchella*, *A. pseudopulchella*)*A. striolata*) clade, with the Western Australian *A. repens* representing the sister taxon to these largely southeastern Australian forms. However, the position of *A. pulchella* differed between the two studies. In Kluge's tree, *A. pulchella* is the sister group to the *A. parapulchella* clade, whereas in our trees, *A. pulchella* and *A. repens* are members of a Western Australian clade.

Speciation Rates

Prior to the Miocene, Australia was primarily a wet-green forested continent (White, 1994). However, beginning about 23 MYA and continuing to the present, Australia has made a dramatic transition to a more arid climate (White, 1994). Such large-scale climate changes could conceivably alter speciation and extinction rates of organisms and, indeed, aridification has been suggested as a major factor driving Australian lizard radiations (Pianka, 1972). Interestingly, the vast majority of extant pygopodid species originated in the last 23 million years even though the group may have existed for at least 37 million years (this study). Plots of lineages through time offer another means of studying the speciation/extinction history of organismal clades. The plot we generated for pygopodids suggests that diversification rates peaked early in the group's history before leveling out over the past 10 million years, perhaps as ecological niches became filled. Results of our relative cladogenesis tests add further support to the idea that this group diversified rapidly early in their history. Our lineages-through-time plot for pygopodids resembles the profile for *Dendroica* warblers by showing initially high diversification rates before a slowdown toward the present (Lovette and Bermingham, 1999; Barraclough and Nee, 2001) but differs from the one for *Ellipsoptera* tiger beetles,

which shows diversification rates increasing, not decreasing, toward the present (Barraclough et al., 1999; Barraclough and Nee, 2001). Although our profile shows at least two possible upturns in speciation rate, one between 23 and 33 MYA and the other between 17 and 23 MYA, any attempt to link these putative upturns to specific environmental events is premature until independent comparisons with other Australian lizard clades can be made. If a number of such profiles are found to agree with one another, this agreement may provide further evidence that Australian lizard diversity has tracked large-scale environmental changes in Australia.

The validity of lineages-through-time plots when the clock assumption is not met is an issue that does not seem to have been addressed. Although some of our molecular characters apparently evolved in a clocklike fashion (i.e., nDNA), the fact that our concatenated mtDNA data failed the clock test still renders our interpretations questionable. In an attempt to assess the sensitivity of our results to the clock assumption, we repeated the analysis using only the third codon position sites of the *ND2* gene (351 sites), a partition of our mtDNA data that arguably best behaves in a clocklike manner. As expected, this partition did meet the clock assumption ($\chi^2_{[0.05]} = 47$, $P > 0.05$). Remarkably, the lineages-through-time plot (not shown) based on this third sites partition was very similar to the one generated using all molecular data (2,079 sites), suggesting that our combined data, despite failing the clock test, remained capable of producing an accurate lineages-through-time plot. The only obvious difference between the two plots concerned the section of the curve between 37 and 27 MYA, whereby the third-sites plot indicated a more rapid buildup of lineages than did the combined data plot, a result that better supports our conclusion of early rapid diversification. However, this difference is clearly attributable to the inability of the third-sites data to reconstruct some of the deeper nodes (C and D in Fig. 11), which resulted in a four-branch polytomy. Although our results are encouraging, additional studies of this problem are needed.

Biogeographic Implications

The biogeography of cool-mesic taxa isolated in the southwestern and southeastern corners of the continent has received considerable attention. Main et al. (1958) examined the distributions of various frog genera in southern Australia and concluded that Pleistocene climate oscillations led to repeated east–west divergences of taxa, resulting in the creation of species whose sister relatives were not to be found in the same refugium. Later, molecular studies of these frogs by Barendse (1984) and Roberts and Maxson (1985) rejected the Pleistocene dispersal model after both studies presented cladistic evidence suggesting that each species' sister relative was to be found in the same refugium, implicating in situ speciation within each region rather than the repeated east–west dispersal scenario of Main et al. (1958). Further, Roberts and Maxson (1985) concluded from a molecular clock analysis of MC'F data that these divergences were far older than Pleistocene times, with most of the frog sister pairs originating 5–14 MYA and one pair arising 26 MYA. The biogeographic pattern reported by Barendse (1984) and Roberts and Maxson (1985) has received considerable support from comparable area cladograms for a variety of other cool-mesic taxa, including birds (Cracraft, 1986) and eucalyptus trees (Ladiges et al., 1987). Roberts and Maxson (1985) suggested that marine incursions into the Eucla Basin, which may have occurred repeatedly over the last 23 million years (White, 1994), could have sundered formerly widespread populations into southeast–southwest daughter populations. Cracraft (1986) also favored a vicariance scenario but instead suggested climate-caused vicariance scenario in which aridification spread southwards to the Australian bight thereby severing east–west gene flow.

Pygopodids offer an opportunity to further evaluate the biogeography of southeastern and southwestern taxa because two clades of these lizards also display this classic biogeographic pattern (Figs. 13, 14f). Our phylogenetic analysis shows a basal split in *Aprasia* yielding eastern and western Australian clades. Similarly, an east–west split is seen between *Delma petersoni* (southeast) and *D. fraseri* + *D. grayii* (both southwest). While our results appear to merely reinforce a previously strong biogeographic pattern, one significant contribution here is that our fossil-calibrated phylogeny permits us to estimate the approximate timing of these divergences, which has to our knowledge only been attempted once before. The basal split in *Aprasia* seems to have occurred 17–23 MYA, whereas *D. petersoni* diverged from *D. fraseri*/*D. grayii* around 12–16 MYA, both rejecting Main et al.'s (1958) Pleistocene divergence model. Estimates of divergence times for several frog sister pairs range from 5 to 14 MYA (Roberts and Maxson, 1985). The variability of these divergence times either may reflect the impreciseness of estimation procedures or may be indicative of multiple vicariance or dispersal events.

Although repeated marine incursions alone or in conjunction with expansion of deserts southward may have ended east–west gene flow for different mesic-adapted taxa at different times, several aspects of pygopodid biogeography strongly implicate climate-caused vicariance, especially aridification, as being the primary agent driving diversification. First, our phylogeny suggests that diversification of the *Aprasia* clade largely occurred within each region. Second, the present distribution of *Aprasia* is highly fragmented, with the majority of populations being allopatrically or parapatrically distributed in temperate-mesic areas along the southern and western margins of the continent and a few relictual populations being found in very remote desert areas (Fig. 12; Kluge, 1974; Wilson and Knowles, 1988; Greer, 1989; Cogger, 1992; Ehmann, 1992; Smith and Henry, 1999). Third, increasing aridity across the vast interior of Australia best explains the relictual distributions of other relatively mesic-adapted species such as *Delma concinna*, which is only known to occur along the west coast between Perth and Shark Bay (Storr et al., 1990), and its apparent sister species *D. labialis*, which is only known from the east

coast near Townsville (Shea, 1987b). Our data indicate that these two species of *Delma* diverged from each other between 22 and 30 MYA.

Similar biogeographic studies have yet to be made for the desert regions that occupy the majority of Australia's land surface. As already intimated, this region supports spectacularly diverse lizard communities (Pianka, 1986), which are derived from all five families of lizards found in Australia. Pianka's (1972) model of arid-zone speciation still represents the only attempt to explain the origins of this diversity and still remains to be rigorously tested with phylogenetic data. Again, pygopodids are an ideal model, because they are diverse throughout the arid zone (Cogger and Heatwole, 1981; Cogger, 1992; Ehmann, 1992) and sufficient phylogenetic information is now available.

Several aspects of arid-zone pygopodids are interesting. First, these taxa tend to have very large geographic ranges, some nearly spanning the continent. Even more exciting is the finding that several arid and semiarid pygopodids are sympatric with their sister species, which has not been previously documented for any lizards found in Australian temperate ecosystems. These sister pairs include *Delma borea*/*D. tincta*, *D. butleri*/*D. nasuta*, and *D. fraseri*/*D. grayii*. Our results suggest these species came into existence only in the last 10 million years, which is consistent with the main mechanism of Pianka's (1972) aridification-diversification model. However, our data do not indicate Pleistocene origins for these taxa as predicted by Pianka, but pre-Pleistocene origins instead. Interestingly, these sympatric pairs also seem to be younger than the three allopatric sister pairs (i.e., *D. australis*/*D. torquata*, *D. molleri*/*D. impar*, and *D. concinna*/*D. labialis*), which conflicts with the general belief that sympatric pairs are older than allopatric or parapatric pairs, a phenomenon previously reported for some Andean birds (Garcia-Moreno and Fjeldsa, 2000).

If these sympatric sister pairs were formed as a result of allopatric speciation, then our data imply that at least some species of *Delma* have undergone recent range expansions. An interesting ecological implication that follows is that we may have identified three new examples of ecological character displacement using phylogenetic information. Although detailed ecological studies of these species have yet to be made, available data indicate that each member of these sister pairs can be found in syntopy with its sister (Kluge, 1974; Jennings, pers. obs.; Pianka, pers. obs.), and preliminary field observations suggest that *Delma fraseri* and *D. grayii* are ecomorphologically divergent from each other (Jennings, unpubl. data). We hope that our results stimulate further studies of these taxa because the possibility that at least some of these species formed via sympatric speciation cannot be ruled out.

Pygopodids are a fascinating group of organisms for a systematic study owing to their modest clade size, endemicity to Australia, and biogeographic distributions and to the availability of at least one well-dated fossil. Although we encountered some problems during our efforts to infer their phylogeny, i.e., unresolved intergeneric relationships and the inability of our outgroups to root the tree properly, we nevertheless believe that we have succeeded in developing a taxonomically well-sampled and robust phylogeny for the group, which in turn can serve as the foundation for future evolutionary studies. Previous investigations into the biogeography of Australian terrestrial organisms have implicated climate change as an important factor promoting the diversification of various groups. Results of the present study are in accord with this general idea, and we provide new insights into the evolution of Australia's temperate biotas.

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